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National Cancer Institute

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

NIOSH

PROCEEDINGS OF THE
SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

SEPTEMBER 9-11, 1981

SHERATON/POTOMAC, ROCKVILLE, MARYLAND

The papers included in these Proceedings were printed as they were submitted to this office.

Appropriate portions of the discussions, working groups, and plenary sessions were sent to the participants for editing. The style of editing varied, as could be expected. To the extent possible, we have attempted to arrive at a consistent format.

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Proceedings were developed from a workshop on the Collaborative Programs of the National Cancer Institute, the Environmental Protection Agency and the National Institute for Occupational Safety and Health on Environmental and Occupational Carcinogenesis.

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PROCEEDINGS OF THE
SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

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PROCEEDINGS OF THE
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PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

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General Program Directions

I. Recommendations

A. In this era of stable or decreasing budgets additional attention needs to be made with regard to supporting projects that are not only good science but also have good management and are likely to yield new information.

B. Studies on occupational and environmental cancer, while focused on cancer should provide opportunities to accrue data and information on other biological responses that may have ancillary relevance to cancer.

C. Collaborative programs between National Cancer Institute, National Institute for Occupational Safety and Health, and Environmental Protection Agency should exploit a multidisciplinary team approach with specific skills and resources in areas of environmental and occupational cancer.

D. Epidemiological pursuits in collaborative projects maintained by the three agencies should endeavor to maximize the development of exposure/response data in studies on occupational and environmental cancer. In occupational studies the agencies should develop and extend the data base on occupational histories and clinical parameters associated with occupational disease states including cancer.

E. The use of a multidisciplinary team approach as well as cancer registries should be fully exploited where exposures to environmental and occupational agents pose an imminent health hazard.

F. To preclude the development of information and data from one program area in isolation from another area, it is recommended that investigators utilizing animal models (experimental area) work closely with their counterparts in epidemiology to maximize the benefits and leads emerging from the laboratory.

G. Recognizing that our counterparts in industry have valuable resources such as employment records, industrial hygiene data and technology and processing data, program staff should concentrate their efforts in extension in liaison and utilization of such resources.

H. Some of the monies in this collaborative effort should be set aside for development of an RFA which focuses on occupational cancer.

Environmental Carcinogenesis

Recommendations

A. Considering the potential for 20-year exposure to a wide array of synthethic chemicals in the environment as a result of accelerated production, epidemiological studies should be concentrated on site specific or age group specific cancer trends.

B. Since epidemiological studies can be dependent on the utilization of a wide spectrum of data bases, it is recommended that collaborative efforts of NCI and EPA exploit and utilize resources of the Social Security Administration, IRS, Census Bureau, and the National Center for Health Statistics in the conduct of epidemiological studies. With fiscal constraints imposed currently this collaboration now is more important than ever.

C. The development of important data bases in the New Jersey study on environmental agents points up the need and utility of such studies in providing a good foundation for further epidemiologic studies where a "rapid response" to public health concerns may arise in the future.

D. In the development and planning of epidemiological studies on environmental hazards, especially at low level exposure, attention should be given in basic experimental studies to more rapid and sensitive procedures. In epidemiological studies ivestigators should look for clinical parameters such as certain metabolites that are "early warning indicators".

E. Since massive efforts cannot be focused on all chemicals in the universe, it is suggested that programs concentrate on priority chemicals, i.e., those of high production or exposure and those with continuous exposure that are structurally related to known carcinogens.

F. The experimental data derived from carcinogenicity bioassay should be screened and evaluated, especially for those chemicals classified with sufficient evidence to provide leads on epidemiological studies of population groups of high cancer risk.

G. Increased emphasis should be placed on detecting interactions between environmental exposures and personal habits (lifestyle factors) such as smoking, alcohol consumption, and dietary patterns or nutrition as they may impact on cancer induction.

H. Since multiple factors may be involved in the induction of cancer, special emphasis should be placed on attempts to elucidate those etiological factors that may increase the incidence of certain types of cancer.

Occupational Carcinogenesis

Recommendations

A. The participating agencies should pool their efforts to support, strengthen and develop epidemiolgical data resources which are located in various federal agencies such as the Social Security Administration, IRS, Census Bureau, and the National Center for Health Statistics, especially during these times of fiscal constraints. The existence of available data must be recognized and exploited to generate and evaluate hypotheses relevant to environmental cancer.

B. As ongoing retrospective cohort studies were being discused the panel felt a growing concern with the consistent problem of "loss to follow-up." In nearly every case this category represented a significant proportion of the total cohort. In one case the fraction lost in this way approached 26%. As long as this difficulty persists it seems unlikely that the resulting studies, where subjected to "worst-case" analysis, will provide convincing evidence except in the unlikely event that relative risks are encountered which approach those for vinyl chloride.

It seems clear that we must, focus our attention on a search for better ways to follow up the members of such cohorts and that this should become a major consideration in our future planning. The NCI/EPA/NIOSH collaboration program should utilize interagency cooperation to attack this problem.

C. The decade of the 1980's is particularly important for the conduct of occupational and environmental cancer epidemiology studies when considering both the great surge in (synthetic) chemical production since the 1960's and the usual latency periods of 20 or more years for cancer to develop. Occupationally related cancer is unlikely to be common event in those under age 45 due to latency consideration. Special efforts should be made to look for occupational factors in those with cancer at age 45 or older. Special attention should be given to examining cancer trends for specific sites and age groups in the coming years due to potential effects from the great surge in chemical production since 1960. These analyses must include people of age 65 and older, again considering latency.

D. Differences in cancer rates between men and women for non-hormone dependent sites should be studied for clues to occupational etiologies given the fact that men tend to have jobs involving greater exposures to toxic hazards.

E. There need to be a greater effort to develop additional control groups for use in occupational epidemiology studies. To the extent that occupational factors contribute significantly to the overall cancer rates, using general population control groups is analogous to looking at the risk of lung cancer in smokers compared to the general population which also includes a fair number of smokers. Search for "unexposed" control groups for environmental studies will also be difficult due to the vast expansion of chemical usage. Collaborative projects should also be developed around certain methodologies that will provide for an appropriate set of comparison rates for spontaneous abortions among working women.

F. Special efforts should be made to evaluate factors contributing to the great increase in lung cancer, particularly in women. More consideration should be given to examining the effects of parental employment upon cancer in children.

G. More attention needs to be given to control technology and personal protective equipment related to the prevention of workplace cancer.

H. More rapid experimental procedures are needed to evaluate the health effects of occupational and enviromental exposures including cancer.

I. Occupational studies should make greater efforts to examine minorities, since these groups often have jobs with the greatest exposure to toxic hazards.

J. Additional efforts should be made to routinely include occupational histories in clinical work supported by NCI.

K. Attention should be paid to all industries where priority chemicals may be a component of the process used and ambient exposure levels should be determined wherever possible.

Planning for Future Workshops

Recommendation

A. In reporting of progress on various projects, the planners of the program agenda should allocate less time for presentation of reports or papers on newly implemented projects. Conversely, more time should be allocated for progress reporting on projects underway for some time or soon to be terminated.

B. It was recommended that Workshops should be scheduled no more than once every two years with the next Workshop targeted for September 1983.

PROCEEDINGS OF THE
SECOND/NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

Wednesday Evening, September 9

INTRODUCTORY REMARKS

Richard H. Adamson
Director
Division of Cancer Cause
and Prevention
National Cancer Institute

John W. Hernandez, Jr.
Deputy Administrator
Environmental Protection Agency

Donald Millar
Director
National Institute for Occupational
Safety and Health

The Honorable James G. Martin
U.S. House of Representatives

OVERVIEW

Herman F. Kraybill
Scientific Coordinator for Environmental Cancer
National Cancer Institute

PROCEEDINGS

CHAIRMAN KRAYBILL: Please come to order.

This is the second NCI/EPA/NIOSH Collaborative Workshop. We had one last year and I think it was a great success.

I would like to, at the start, thank all of the committee members because without their help this program, and none of the planning, would have been possible. And we want to express our appreciation to Dr. Bridbord, Dr. Leidel, Dr. Cameron, Dr. Farland, Dr. Galbraith, Ms. Blackwood, -- that "farmer Kraybill," forget him -- also Dr. Tom Mason for planning most of this meeting and what you see before you.

We had some misgivings about a week or two ago when we were getting a lot of calls; we know this room holds at least 250, I believe, but we got a little nervous that we would have an overflow crowd. You don't see it here tonight, but I guarantee you're going to see it tomorrow and Friday because we expanded this meeting from last year. Last year, we only had the Federal representatives, no contractors; but this year we have the representatives from the three agencies here, including the contractors who are working on these projects plus representatives from trade associations, the labor unions, and I don't know if there will be any press here or not, but the coverage is quite wide.

So tonight I must apologize, Dr. Adamson and Congressman Martin, for the attendance we have here but don't gauge it by what you see here right now because we're going to have a big crowd tomorrow and Friday.

CONGRESSMAN MARTIN: You just let the word out that I was going to be here, Herman, and that's what happened.

CHAIRMAN KRAYBILL: Oh, my; I can't top that one.

Anyway, I think we should start immediately because we are late, and I would like to call on Dr. Richard Adamson, who is Director of the Division of Cancer Cause and Prevention from the the National Cancer Institute, to express the views of the National Cancer Institute. Dr. Adamson.

INTRODUCTORY REMARKS

Dr. Richard H. Adamson
Director
Division of Cancer Cause
and Prevention
National Cancer Institute

OPENING REMARKS

Richard H. Adamson, Ph.D.
Director
Division of Cancer Cause and Prevention
National Cancer Institute

Good evening. Welcome to the Second NCI/EPA/NIOSH Collaborative Workshop. This workshop is timely -- today Congress has returned from a recess, the EPA and NIOSH budgets have been reduced for FY82, a potential move is being contemplated for NIOSH, and the management of the NCI, is undergoing close scrutiny.

The NCI collaborates with various agencies by a variety of mechanisms including interagency participation on committees, membership on task forces in response to legislation, participation at various legislative and regulatory hearings, various informal mechanisms, providing specific advice, epidemiological or research data, and of course by pass-through money.

This workshop on environmental and occupational cancer represents another mechanism for active collaboration on projects between NCI, EPA and NIOSH. In these times of budgetary reductions and constraints, NCI will work with NIOSH and EPA to maximize collaboration with minimum investment. In this collaboration, support funds come from NCI and 50 percent of the projects are NCI-initiated and -monitored projects which are endorsed by the other participating agencies. However, the projects developed and planned by NCI, NIOSH and EPA in the areas of environmental and occupational cancer will receive, beginning this September, concept approval by the Board of Scientific Counselors of the Division of Cancer Cause and Prevention project by project rather than as a program in total. Following this approval, they will receive review and approval by contract review committees.

Under budgetary limitations, NCI has still exhibited a spirit of cooperation with EPA and NIOSH. The future will dictate to what degree we can sustain these ventures. Nevertheless, it should be appreciated by our colleagues at the other agencies that we must work together now to critically appraise all projects in both programs to achieve high quality relevant investigations from which all may mutually benefit. One of the major goals of this workshop is to critique current performance and render advice as to what future directions this program should pursue.

To track the progress of these interagency projects, contract progress reports and annual reports are required. This workshop and the Proceedings developed from this workshop are, additional means for assessment of program accomplishments. Concerning the future, a cycle of two years may be adequate for workshops but contract progress reports and annual reports will still be required.

We look forward to a most successful collaborative program since we should now be experienced in responding to mutual needs in mounting projects that are relevant, scientifically sound, and well managed.

Thank you

CHAIRMAN KRAYBILL: I was gratified to hear my Chief's remarks, and I think they were quite appropriate.

I would like to emphasize one point that he made, and this is an admonition. You recall at last year's meeting we had some superventilation: I hope we can reduce some of that. I know it's democratic to let people get up and hold forth for 10-15 minutes, but I would say rather than talk about each paper, per se, do what Dr. Adamson said here, critique the projects: what do you think of the quality of the projects, and what kind of projects should we have in the future, and which way should we direct our projects?

I think if we have a good program we have good projects. I think it will make the Cancer Institute far more comfortable in collaborating. Am I correct?

DR. ADAMSON: I think that's correct.

CHAIRMAN KRAYBILL: Now our next speaker will be from the Environmental Protection Agency, Dr. John Hernandez. Dr. Hernandez, please.

INTRODUCTORY REMARKS

Dr. John W. Hernandez, Jr.
Deputy Administrator
Environmental Protection Agency

Opening Remarks by Dr. Hernandez to the Second Annual Collaborative Workshop

It is with great pleasure that I am here to participate in the opening of the Second Annual Collaborative Workshop on Environmental and Occupational Cancer. This effort is an example of the type of collaboration we will need more of in the future if we are to obtain the greatest possible return from the limited Federal research resources available.

In the past, each Agency generally proceeded solely within the framework of their mandates. There were sporadic cooperative efforts, but these were mostly by individual scientists who collaborated informally with colleagues in other agencies. There were also times when individual Agencies "contracted" with other Agencies for specific tasks. The problem was that these efforts were often narrow in scope and too specific to foster continuation.

As time passed, it became evident to our scientists and political leaders (1) that the talents in the various Agencies were not being utilized to the fullest; (2) that we could improve our overall scientific achievements by improving interagency cooperation, and (3) that we had many scientific and regulatory problems which could best be solved by full and formal cooperation.

From this realization sprang such cooperative efforts as were embodied in the tasks of:

a. the Task Force on Environmental Cancer and Heart and Lung Disease
b. the Interagency Technical Committee on Heart and Lung Disease
c. the Interagency Regulatory Liaison Group (IRLG)

to mention just three EPA interagency cooperative efforts.

In keeping with this group of cooperative efforts are also the endeavors we will hear about in this, the second, collaborative workshop on environmental and occupational cancer. All these efforts, though young in age, have been important and have already resulted in many good things. The results we have obtained would have taken longer and been more costly had we pursued only our own narrow interests.

Through the Task Force on Environmental Cancer and Heart and Lung Disease the annual reports prepared for Congress point out progress we have made, as well as problems we still face. We present a common stance in our continuing effort to inform the public and Congress about environmental causes of cancer and heart and lung disease. This Task Force has also produced workshops which educate the various health professions on the environmental causes of heart and lung diseases and devised better ways to educate the various health professionals.

Together we utilized our talents to resolve the educational gaps which existed and then, together with the academic and industrial communities, set about to accomplish the necessary education of health professionals.

Through the joint effort of scientists, lawyers, engineers, and others, common testing strategies, common testing guidelines, and other combined common needs are in the process of coming to fruition. Not only will this help simplify the regulatory requirements stipulated by Agencies such as EPA, but it will also save industry countless dollars by their knowing what tests will be needed by the various Agencies, how to conduct them, and most of all that a test protocol followed for one Agency will be accepted by another Agency.

Another benefit derived from these cooperative efforts is that we in EPA have had to reevaluate and think deeply about our own work, its direction, quality and where it will lead us. With the realization (1) that we cannot achieve our individual Agency goals alone, in a way which is efficient, economical and comprehensive, and (2) that cooperation in our varioius specialities can only be strengthened by pooling our common talents into an effective effort where our interests overlap.

A sound beginning has been made in all these areas I have mentioned. This was evident from the results presented during the first workshop of this collaborative group and from what I have indicated above. I am sure our expectations will be verified by what we will hear later in this second workshop.

From these cooperative efforts, which have been in effect for only 2 to 3 years, the realization has been strengthened that we must continue and build on the cooperative successes made to date. Efforts such as this provide a forum through which scientists in the various Agencies become familiar with the programs of other Federal Agencies and provide research planners with information which helps eliminate duplication. A mechanism is also provided through which a regulatory Agency can make its needs known to a basic research organization and, of course, the program provides resources to accomplish tasks pertinent to the missions of the concerned Agencies.

It is of paramount importance that EPA policy-makers have scientifically accurate information available when making regulatory decisions. For this, we rely on research and development. However, EPA research and development resources are limited and those available are directed to meet needs which are not or cannot be met by other Agencies. It is hoped that through mechanisms such as the NCI/EPA collaborative program on environmental carcinogenesis that EPA will receive support in scientific areas in which we have limited or no capabilities. Work in areas in which EPA has established resources should result in greater success than can be attained by either Agency working alone.

A review of the second annual report of projects supported by the NCI/EPA collaborative program reveals that this objective is being partially met in epidemiology. EPA does not have the epidemiological resources that are at times required to fulfill our mission. In the future, we will rely more heavily on the Department of Health and Human Services for epidemiological support and the NCI/EPA collaborative program on environmental carcinogenesis will play a significant role in this regard.

I was also pleased to learn that this program has played a significant role in tying together research efforts by various Agencies evaluating carcinogens, mutagens, and teratogens in aquatic animals. Collaboration in this area has resulted in a product pertinent in the missions of each Agency and has required less resources than would have been necessary in a non-collaborative effort.

I am confident that the exchange of information and ideas that will occur tomorrow and Friday will effectively enhance work in these and other research areas of mutual interest to NCI and EPA. I look forward to seeing the proceedings and learning of the achievements resulting from this workshop.

Thus, we have a great deal of work ahead of us which must be done more efficiently and effectively if we are to cope with diminishing resources in funds and trained scientists.

The only recourse seems to be that we, in the future, must increase and strengthen our collaborative efforts. We in EPA are prepared to do this to the best of our ability.

It is my understanding that the working relationships between the NCI Director and the Director of the Division of Cancer Cause and Prevention and his staff and EPA staff has been excellent in the past. I would like to express my appreciation to Drs. Devita and Adamson and say that I look forward to a similar relationship in the future.

Thank you and good luck in your work in this workshop.

CHAIRMAN KRAYBILL: Thank you, Dr. Hernandez.

Speaking of the Task Force Report, I think it's time that we show our appreciation because we received the support from Dr. Millar, Dr. Adamson, and you, Dr. Hernandez. All of the agencies signed off, and we appreciate that.

Our next speaker presenting the views for the National Institute for Occupational Safety and Health, and the Director of the Institute is Dr. Donald Millar.

Dr. Millar.

INTRODUCTORY REMARKS

Dr. Donald Millar
Director
National Institute for Occupational
Safety and Health

PRESENTATION OF DR. DONALD MILLAR
NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH

DR. MILLAR: Thank you.

I appreciate the opportunity to be here, as well. I am newly appointed over at NIOSH, and must say the first day at the office I pulled a drawer open in my desk and there was a bullet in there, and I looked at that. You know, I'm sort of from down in the country, and this puzzled me a great deal. I stood there and pondered it for a number of minutes trying to figure out just what this was. One of the staff people was sitting over at the side and said, " Look at him, he dosen't know whether to bite it or shoot it."

But I am happy enough to be part of this particular program. I think this is a collaborative effort that we can all be quite quite proud of.

If you look at the causes of death, of premature death of Americans today, the top three causes of premature death are heart disease, cancer, and accidental injury. And we don't have data that are that good for the workplace, but I think it's safe to say that in terms of the contribution of occupation to that we are probably looking at cancer and accidental injury as the real role of the work place in premature mortality. So this collaborative effort hooks right into what is really a major health problem in the country.

I am reminded that, for instance, last week one of the bigger issues we were dealing with in the media were the issues surrounding what the government's responsibility is in informing workers that are known to be exposed to high risks of carcinogenic compounds. This issue is far from resolved and it will be on our plate at NIOSH for quite awhile; but I think it emphasizes the fact that the issues that are being dealt with here are indeed important issues with very considerable relevance to the health of the public.

We are very pleased to be part of this effort in another sense, as well, because we have been very direct benefactors since Fiscal Year 1976. In fact, NIOSH has been provided $17 million to carry our research related to cancer and the workplace, and as a direct consequence we have been able to complete, or initiate between 60 to 70 projects. These projects have materially assisted us in our program to identify and reduce the risks of carcinogens in the workplace, and I can only say that I'm sorry that the realities of the recent past and immediate future suggest that this is a declining figure because we have found it very useful.

We have 24 projects continuing from Fiscal Year '80, and we had planned to engage in four new ones in Fiscal '81. Only three of those actually will have been initiated in Fiscal '81. But indeed, we consider this an important part of our ongoing research activity.

I had intended to review some of the highlights with you, but I think I will not do that because many of these are covered in the program itself.

I would, instead, like to refer briefly to what I think are some of the advantages that NIOSH brings to this collaborative effort. I think there are some things that we can do, and some advantages that we have that perhaps are not found elsewhere.

We do have a thing called the right of entry into the American workplace, which is assured us by law, which I think enables us to engage in some investigations which would not otherwise be available to people in this field. Also, I think we have a very unique combination of the sciences and skills of epidemiology and industrial hygiene so that we do have the opportunity to look at dose-response relationships perhaps in a way that is different from other settings.

We also have an interest in control technology, which means that we can, in fact, test and evaluate methods of intervention in the workplace setting to determine what, indeed, the effects or reducing levels of exposure might be over the long haul.

And finally, I think in my immediate prior assignment as Director of the Center for Environmental Health at CDC, I became very aware of the fact that the information that NIOSH generates in the workplace has very great relevance to the population as a whole in a way that would not be possible without investigations conducted in the workplace.

So we think we have an important set of assets to contribute to this collaboration, and we certainly look forward to being active in it in the future.

We recognize, as Dr. Adamson has already referred to, that times are getting tough, and that the competition for scarce dollars in research will become ever more keen, at least in the foreseeable future. We think that indeed, this, as he suggested, is an even more pertinent reason for collaboration and cooperation than we have had in the past, and we would very much like to see this collaboration intensified. We have some specific notions on that. We would like, for instance, to have more joint project officers involved. We would like to continue joint program planning. We would like to contemplate actual exchanges of scientists between NIOSH and the other agencies involved; and we would also like to explore, perhaps with other aspects of the National Cancer Institute, programs that might be interested in collaborating with us in other areas, specifically control technology and protective equipment.

So without further ado, just let me say that we are happy to be part of this. It is an important part of our program, and you can rest assured that we will be eager to participate in the future.

CHAIRMAN KRAYBILL: I particularly appreciate your remarks, Dr. Millar. One of the things we tried to stress, and I'm glad to hear you say it, in a collaborative program we must -- let me emphasize must -- have project officers on both sides, and I don't mean just in print, but in the true spirit of collaboration that they talk to each other.

I must admit that I don't know as much about the NIOSH program, and I was reminded of that when Dr. Adamson called me one day and said something about it, and I said, "Well, it's in good hands." And I think Dr. Cameron has done a good job of coordinating and working with the NIOSH group, and I think we've seen some big changes in that collaborative program, and a lot of thanks go to you, Dr. Cameron.

The next speaker will be the Congressman. When I talked to Congressman Martin, or Dr. Martin, I said, "You know, you've been at meetings before that we had, and we think a lot of you. You've done a superb job; but I wonder if it wouldn't be a good idea to have some representatives from both parties of the House, so to speak."

Well, I don't know how he took that, whether he nodded approval to it, but I said, "Maybe we ought to have two Republicans--Congressman Martin is a Republican from North Carolina--and two Democrats. So we invited two Democrats and two Republicans, and it happens that the two Republicans are both Ph.D.'s, Dr. Ritter, and Dr. Martin. I don't believe Mr. Brown is a scientist.

PARTICIPANT: Yes, he is.

CHAIRMAN KRAYBILL: So we were trying to show that we weren't just all on the Republican side.

All right, without further ado then, I'd like to introduce Congressman James G. Martin, from the State of North Carolina, formerly Professor of Chemistry at Davidson College. And believe me, fellows, he knows what he's talking about. He's a scientist like we are, so don't try to pull the wool over his eyes. Dr. Martin--Congressman Martin.

INTRODUCTORY REMARKS

The Honorable James G. Martin
U.S. House of Representatives

STATEMENT OF
CONGRESSMAN JAMES G. MARTIN
AT
2ND ANNUAL WORKSHOP ON NCI/EPA/NIOSH COLLABORATIVE PROGRAM ON ENVIRONMENTAL AND OCCUPATIONAL CARCINOGENESIS
SEPTEMBER 9-11, 1981, ROCKVILLE, MARYLAND

I do enjoin you, Dr. Kraybill, let's go easy on the farmers. They've got enough problems these days.

I do want to thank you for the invitaiton. I'm a little sorry that my three colleagues weren't able to be here this evening. Normally, I'm not in a position to be able to speak for my colleagues. We do have both partisan and ideological divisions across the House and the Senate. If you thought you had it right the way the House is going to act, you still have the Senate to contend with, and, of course, there have been some profound changes there. Nevertheless, my colleagues are not here and so tonight I am going to presume that I will be speaking for them and if they don't like it they should have been here.

I had the privilege a couple of months ago of participating in a similar, somewhat related seminar, or workshop on cancer and heart and lung disease. Both the preparaiton for that, and also looking to see what the minutes indicated had transpired during the session, compelled me to read a series of reports that, let's be honest about it, I might otherwise have missed. Of course, that is the beauty of what you are doing, and being asked to do with these collaborative efforts, the workshops, the projects, the programs that you are undertaking. It not only brings people together from different disciplines, it even beings people together from the same disciplines, but who are with different agencies, and different program goals.

I think it helps all of you if you are better informed, each of what the other is doing, whether it be the National Cancer Institute, or the National Institute of Occupational Safety and Health, or the Environmental Protection Agency. I believe this is what Congress intended in 1976, and then in 1977 in providing for the set-aside of funding for these joint ventures. Frankly, I'm amazed that Congress had that good insight.

You know, if you are familiar with the legislative process itself, then you know the old story about how it's very much like the process of making sausage. The more you know about how it's done the less appetite you have for the product that comes out of it.

I don't think I have to tell you the importance of this collaboration. I have mentioned the cross-fertilization aspect of it, the sharing of personal insights and information, the emphasis on coordination rather than the duplication of inquiry that results from not knowing what's going on in a related camp.

Surely, each of the agencies represented here this evening has its own different legislative perspective. You have different regulatory objectives; you have different guidelines, different grounds, different standards that you've become attached to in the past, and yet it's clear that the incidence of cancer, whether arising out of occupational exposure or environmental exposure, or personal day-to-day habits, whether living, working, or playing, or what it might be, is equally of concern to us as a nation and to you as responsible agencies in these fields.

These, I think you will agree, are exciting days for the kinds of studies that you are undertaking because people are beginning to unravel and to reveal some of those innermost secrets of the human cell, and of cells of other animals as well. We are beginning to learn a bit more about the DNA damage and repair functions, about the immune functions, about the role of metabolism both in changing an otherwise harmless and useful substance into a dangerous one, or the reverse, detoxifying.

The role of promoters, of irritants, of cocarcinogens, or precarcinogens, and even the psycological effects seem to be very important factors.

It was just a few years ago that Congress faced a somewhat bleaker uncertainty about cancer cause and effect, mechanisms, and processes. It enacted the Delaney Clause, an absolute zero risk standard, which said that any artificial additive to the food supply would not be permitted as safe if it were found to cause cancer in any reliable experiment. This limitation was imposed without regard to dose effects, without regard to negative evidence, without regard to benefits, without regard to relative risk, without regard to anything except one reliable line of evidence that the substance could be dosed to a level that would produce tumors in a reliable experimental framework.

Today people are beginning to raise questions about this absolutist standard and about efforts to extend it to the workplace and to the environment. We challenge the policy that would result in banning from the workplace and environment all chemicals except those which are such toxic poisons that you can't dose the experimental animals at a high enough level long enough to produce tumors.

Today people are beginning to ask questions about the regulation of low doses of hot substances, and higher doses of relatively weak carcinogens. They are beginning to ask questions about the meaning of massive maximum tolerable dose, and about how you translate that to the effect of doses that humans, even those who work with the substances at a higher exposure, are actually exposed to today, not just 20 years ago in the shipyards, but today in various ways. They are asking questions about what proper regard should be given to offsetting considerations of the cost of removal, of the availability of alternative substances, of the benefits both from a health standpoint, a nutritional standpoint, even the standpoint of the very important public personal responsibility of being able to make one's own choices about one's lifestyle.

I think we are going to be better equipped to address these questions legislatively over the next few years as this science base continues to evolve, and as it continues to be shaken through the scientific mechanisms that you deal with; and as it continues to be shared so that not only do you have the benefit of other insights, but you have the benefit of critical questions that are raised perhaps from outside of your agency that might not have been raised from within.

Today people are beginning to ask more questions about relative risk concepts as opposed to absolute concepts. To answer these questions we must rely on the work that you are doing, such as studies of the statistical base that is available from Public Health statistics, the epidemiological studies that one of your sessions will be devoted to - I believe that's tomorrow's subject - as well as the use of animal testing and cultures for predictive studies that can help us in less expensive ways to determine which substances are carcinogens. We need a better understanding of the mechanism of various classes of carcinogens; how potent they are, and what that has to say about the levels that humans are actually exposed to.

Your working sessions tomorrow and Friday are the heart of this collaborative process. I have had an opportunity to scan the preprinted abstracts that you were kind enough to provide me with. It's obvious that you have a good program. I couldn't help but entertain some curiousity though about the last page where it shows that the program subject for 2:30 p.m., right after the break, is "Coffee and Concurrent Discussion Groups on Future Directions." I wasn't sure whether the subject matter was the toxicity of coffee, or whether the beverage was merely a mode of stimulating discussions on some other concern that you might have. It will be interesting to see how that all works out.

So I commend you, and even your colleagues who couldn't leave their laboratories this evening to be here, but who should be with us tomorrow and Friday. What you are doing here in these sessions, and in the funded projects themselves, will be the proof of whether the legislative judgment was wise, and whether the purpose of that judgment can be fulfilled through these kinds of mechanisms and if so, how to expand them so as to include a wider audience.

I will say to you that personally I am impressed with the quality of the reports that have been issued from previous sessions of this and related groups, and I hope that you will continue to benefit from the opportunity, from indeed the direction that has been given for these projets, and these collaborative programs.

Thank you very much.

CHAIRMAN KRAYBILL: Thank you very much, Dr. Martin. I was reminded about the first Congressional hearing I attended back in the 50's, and I was scared. The Surgeon General of the Army said, "We will fly you in from Denver and you have to make the presentation for us." So they got me up like a student and drilled me, and drilled me, and I had to rehearse everything I said, and they checked all my charts and everything to be sure they were letter perfect and that I said the right thing.

What I'm stressing here in Dr. Martin's case because he is a scientist he is aware of our program. I would recommend to you that you read some of his papers. He has presented papers at the American Chemical Society meetings, and he has written some good philosophical treatises, I would say, in carcinogenesis.

OVERVIEW

Dr. Herman F. Kraybill
Scientific Coordinator for Environmental Cancer
National Cancer Institute

OVERVIEW

H. F. Kraybill, Ph. D.
Scientific Coordinator for Environmental Cancer
Division of Cancer Cause and Prevention
National Cancer Institute

The collaborative programs on environmental and occupational cancer, conducted through interagency agreements between the National Cancer Institute, the Environmental Protection Agency and the National Institute for Occupational Safety and Health, had their inception in 1976 and 1977. The U. S. Congress and the Office of Management and Budget noted that public attention was drawn to the number of potential environmental and occupational carcinogens. While each agency, through legislative authority and program planning, was developing research programs to identify and classify the various environmental and occupational carcinogens, it was at that time deemed advisable by the U. S. Congress and the Office of Management and Budget to require some interagency collaboration to more effectively carry out the national goals toward prevention of cancer. This was not surprising in that the U. S. Congress has traditionally encouraged close collaboration and coordination between Federal agencies in mutually supportive roles to prevent duplication and reinforce each agency's goals. Broader utilization of staff capabilities, expertise, and facilities also enhance overall performance.

The original request imposed upon the National Cancer Institute stipulated the use and sharing of NCI funding--a provision that dictated that neither agency could utilize the funds unless the program and projects were mutually sponsored and worked on conjointly. Since this earlier period, the National Cancer Institute has elected to maintain this spirit of cooperation and collaboration with EPA and NIOSH. While the NCI/NIOSH program has been supported on a year to year basis, the interagency agreement between NCI and EPA was originally approved from June 22, 1978 to June 22, 1984. This document carried the signatures of the Director of the National Cancer Institute and the respective operational units at EPA, specifically the Office of Toxic Substances and the Office of Research and Development. The dollar ceiling imposed on both collaborative programs was $4 million each with no inflationary escalation in funding since the original initiation of the program. It should be emphasized, however, that only part of the total funding, about 50 percent in the case of the NCI/EPA collaborative program, is transferred to EPA. The other 50% is used to support NCI initiated projects. There has been a diligent effort with both programs to have project officers from the initiating agency with a coproject officer from the other agency to insure that each project reflects the ultimate in mutual planning, direction and supervision so that the maximum interests of each agency are fully realized. The coordinators and advisory groups involved in the supervision and guidance of each program are continuously striving to improve the mechanisms of collaborative planning and supervision.

Under both interagency agreements all new and/or proposed projects and extensions of projects requested must receive a "concept" review by the Division of Cancer Cause and Prevention's Board of Scientific Counselors whether they are NCI, EPA or NIOSH initiated. Prior to such a review by the Board, or concurrently with the Board review, each project is reviewed by Advisory Groups consisting of senior program staff from NCI and EPA for the NCI/EPA Collaborative Program. A similar review mechanism exists for the NCI/NIOSH Collaborative Program. This review by the Advisory Groups is designed to assess the overall merit and technical relevance, need and priority of each project which is proposed.

The number of projects under each collaborative program vary from year to year dependent upon new projects introduced, projects terminated and, more significantly, on the magnitude of funding per project. For example, the NCI/NIOSH Collaborative Program in one year had 71 projects whereas the NCI/EPA Collaborative Program had 20 projects in a specific year. Over three years, a total of 35 projects have been supported under the NCI/EPA program.

As to monitoring of these collaborative programs, each project officer along with the coproject officer requires progress reports and makes site visits, periodically, to discuss current progress and future directions which are consistent with original workscope authorized in the approved contract. Biannual and annual reports are required and, for the second year now, under the NCI/EPA Collaborative Program, the Annual Report consisting of progress reports for each project has been published and disseminated in 300 copies to NCI and EPA staff and representatives from other agencies. Another instrument for monitoring the achievements in both these programs is to have workshops and this is precisely why we are here today. These workshops facilitate analysis of programs and evaluation of projects as well as provide advisory viewpoints as to future emphasis and directions. We hope that this second workshop will again succeed in meeting these objectives. Additionally, the Proceedings which are published from this workshop and the previous one are another resource for all concerned to trace the achievements and the overall performance of these cooperative projects.

In our first workshop we had participation and interfacing among program staff and project officers from NCI, EPA and NIOSH and a few contractors. This year we have expanded the participation to include most contractors and representatives from other agencies plus invitees from trade associations, universities and various research institutes and members of the general public. We hope this orientation and session discussions by participants will be useful. The concurrent sessions scheduled during the two days of this workshop should provide an opportunity for responding to the objectives we set forth in the conduct of this workshop.

In essence the areas of emphasis in either of the two collaborative programs are essentially encompassed under four categories as follows:

a) Information/Monitoring and Data Resources
b) Experimental and Mechanistic Studies
c) Methods Development and Approaches
d) Epidemiological Studies

We hope that all participants at the workshop will identify with these areas and select the concurrent session they will attend. Discussions on methods development/monitoring and information/data resources will be included under concurrent sessions A and B although the titles specified in the agenda do not include specific reference to those areas.

For future directions and planning, we would appreciate your candid remarks relevant to this workshop such as: a) Frequency of workshops (12-18 months, etc.)? b) Value of agency interchange of views on various projects? c) Format of agenda both in coverage and time alloted per presentation? d) Length of workshop - 2 or 3 days? e) Type of participation by outside groups? f) Other miscellaneous comments on the workshop.

We look forward to a successful workshop and, through your participation and support, we believe that this objective will be achieved. The Organizing Committee wishes to express their thanks and appreciation for your efforts in this second workshop. We have a compendium of abstracts available and, in due course of time, Proceedings of the Workshop will be made available.

CHAIRMAN KRAYBILL:

We hope the orientation and session discussion by participants will be useful. The concurrent sessions scheduled during the two days of this workshop should provide an opportunity for responding to the objectives we set forth in the conduct of this workshop.

In essence, the areas of emphasis in either of the two collaborative programs are essentially encompassed under four categories as follows: (a) information monitoring and data resources, (b) experimental and mechanistic studies, (c) methods development and approaches, and (d) epidemiological studies.

Now, I find in looking over the final program that we made some slight slip-up. Last year we had these four categories encompassed in concurrent sessions, so I would plead with the session chairmen to be sure to encompass these four categories in their sessions and discuss it quite freely because we only have listed here experimental studies and epidemiological studies. So you will have to cover methods development and approaches, and information monitoring and data resources.

We hope that all the participants at the workshop will identify with these areas. You have the chance if you haven't already, to indicate what session you want to go to, and select that concurrent session.

Discussions on methods development, as I said, and information resources will be encompassed within sessions (a) and sessions (b).

For future directions and planning we would appreciate your candid remarks relevant to these workshops. Frequency of the workshop -- and Dr. Adamson, I think helped us out here. He indicated 18 months or two years, so I think that makes sense, so that's what we will titrate toward in the future.

As to format of the agenda, both in coverage and the time allotted for presentation, the Committee drew this up. So if you think two days is not long enough, we should have had it for three days; if you think ten minutes is too short, that fifteen minutes would be better, we would welcome your comments, please, because we can benefit from your suggestions.

The type of participation by outside groups, how free should we be about this? Any other comments that you may have on the workshops, would be welcomed

The Organizing Committee wishes to express their thanks and appreciation for your efforts in the second workshop.

We have a compendium of abstracts available, and in due course of time the proceedings of the workshop will be made available. We have somebody helping us this year.

We are going to have it all taped tomorrow and you will get your version for you to edit. Please get it back to us promptly.

So with that I'll say that concludes our introductory session for the workshop this evening and we will see you here tomorrow morning bright and early.

Congressman Martin, if you'd like to join us Thursday and Friday, I know you have a very busy schedule, but if you want to drop in and see how it's going why we'd sure be glad to have you.

CONGRESSMAN MARTIN: Thank you, sir.

PROCEEDINGS OF THE
SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

Thursday Morning, September 10

Epidemiological and Statistical Session

Session Chairperson:
Dr. Joseph F. Fraumeni, Jr.
National Cancer Institute

PROCEEDINGS

DR. KRAYBILL: May we come to order please? I don't see the 250 to 300 people we were supposed to have but I guess some will be coming in. I would like to make a few announcements before Dr. Fraumeni starts his session because the chairmen and the concurrent group session chairmen have asked about how we are going to handle the communication system on making an analysis of the various programs and how we summarize the main points that are brought up concerning the projects, that is, whether these projects are meeting their objectives and what sort of modifications of the projects might be in order. That is the first point.

A tougher issue to deal with is what future directions we can take, particularly because some projects will phase out this year; some will phase out next year. And what areas have we not covered in the area of environmental and occupational cancer; what kind of projects should NCI, EPA and NIOSH have that will bring to bear upon the program?

This puts a heavy responsibility on the session chairmen, as well as the chairmen of the concurrent sessions. So we devised a scheme here, either for better or worse, I don't know, that may come to grips with this problem. We have outlined more or less a pyramiding system for the chairmen, the chairpersons, of the concurrent sessions.

We ostensibly have six chairmen for both days, and I can read those off: Dr. Fraumeni, Dr. Burton, Dr. Bridbord, Dr. Yodaiken, Dr. Morris and Dr. Saffiotti. And then the plenary session chairpersons are Dr. Marland and Dr. Adamson.

Now, I was advised this morning that a more efficient mechanism might be to have the concurrent session chairmen, that is A and B for both days, synthesize from their sessions some of the salient points. There may be only two or three major points, so be it. The chairmen of concurrent session A and B would then funnel their remarks to the chairman of the plenary session. Now I have thoroughly confused you I guess.

Let me read them off: Dr. Spirtas, Dr. Yodaiken, Dr. Mason, Dr. Galbraith in the area of epidemiology; Dr. Cameron, Dr. Weisburger, Dr. Cooper and Dr. Farland, in the experimental area, would have to take some pretty good notes and make a summary or synthesis of what was discussed and what went on in their session.

Is there any question? Do you understand the system because a lot of people asked last night how it would be done. Do I see any hands?

All right. That's the way it will be done then. For today's session at the end of today Dr. Marland will be the plenary session chairman and if you prefer to meet with these people today during the coffee break or at lunch hour, you can talk to those. You have three chairmen and you have four concurrent session chairmen.

I want to make it democratic if you can devise a better scheme, please do so. But that system might work.

The second comment is, we've been asked that all you people, when you get up and you make a comment, please give your name and affiliation. If you don't give your affiliation, we have the registry and we can get it from that. But please give your name because that is the only way we can record who said what for our proceedings. Then you will get a copy of the transcript to correct your comments because what you say orally doesn't always look like what you'd like to see in print. So you have a second cut at putting it down the right way. Thank you very much.

DR. FRAUMENI: The first session then deals with epidemiological and statistical matters. We are fortunate to have a strong lead-off batter in Linda Pottern who will present the study on lung cancer in communities with nonferrous smelters. The co-project officer is Dr. Carl Hayes, and the project has been done in conjunction with Lehigh University.

PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

A Case-Control Study of Lung Cancer Near a Zinc Smelter: Preliminary Findings

Linda M. Pottern
Linda E. Morris
William J. Blot
B. J. Stone
Joseph F. Fraumeni, Jr.

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A Case-Control Study of Lung Cancer Near Zinc Smelter: Prelimary Findings

Linda M. Pottern, Linda E. Morris, William J. Blot, B.J. Stone, and Joseph F. Fraumeni, Jr.

Introduction

A case-control study was undertaken in an industrialized area of eastern Pennsylvania, to evaluate the relative contributions of occupation, smoking, and environmental pollutants to lung cancer risk. Located in this area are a large zinc smelter and a major steel producing facility.

Methods and Data Collection

Subjects for the study were identified from a computerized mortality tape supplied by the state of Pennsylvania. The cases consisted of Northampton and Lehigh County residents who died of lung cancer during the years 1976-1977, and Carbon County residents who died of lung cancer during the years 1974-1977. Those subjects who were diagnosed prior to age 30 or after age 79 were excluded. Controls were randomly selected from other causes of death excluding lung diseases and suicide, and were matched to the cases on year of death, usual county of residence, sex, and age at death (within five years). All cases and controls were white.

A total of 447 lung cancer cases and an equal number of controls were identified. Information was obtained from the next of kin of 430 cases (96.4%) and 426 controls (95.3%). Field operations were conducted through a support service control with Lehigh University.

The medical records of the lung cancer cases were reviewed for verification of the diagnosis, including the histologic type, site of the tumor, and methods of confirmation. Thirteen cases were found to be incorrectly classified. Two additional cases with questionnaire responses of poor quality, and 4 controls who had a history of lung cancer were deleted from the analysis. The final study population upon which the analyses were based consisted of 837 study subjects.

Preliminary Results

Males represented 80% of the study population (335 cases and 336 controls) and females 20% (81 cases and 85 controls). The average age at death for males (63 years) was greater than that for females (61 years) and similar for both cases and controls. The county of usual residence as stated on the death certificate was Carbon 19%, Lehigh 43%, and Northampton 38%. Male cases had less post high school education than male controls, whereas female cases graduated from high school and had a post high school education more often than female controls. This is in agreement with social patterns in which educated women tend to smoke more than women with less education while the reverse seems to be true for men.

The preliminary analysis focused on three factors: cigarette smoking, occupational exposure, and residential exposure which may influence the risk of lung cancer in this three county area. Cases started smoking at an earlier age than controls and females started smoking approximately 5 years later than males. Males showed a clear trend of increasing risk of lung cancer with increasing number of packs of cigarettes smoked per day. Risks for females were also elevated, although they showed a less consistent trend with regard to the amount smoked.

To examine work exposures for males, relative risks were calculated according to whether they were "ever" employed in an industry and whether it was their industry of "usual" employment. For "ever" employment, the risk was elevated for the steel, smelter, transportation manufacturing, and chemical manufacturing industries. For "usual" employment, the risk was elevated for the following industries: construction, steel, manufacturing, and smeltering. The greatest risk was seen for those subjects who worked 15 or more years in either the steel or smelter industry. Future analyses will simultaneously take into account the effects of occupation and smoking.

A lifetime residential history was obtained on each study subject. Each residence reported within the three county area was assigned grid coordinates. Environmental measurements were taken in the three county area and also were assigned grid coordinates. Future analyses will involve calculating lung cancer risk according to distance from the smelter and the steel company, and according to the levels of various pollutants in the air measured by the state of Pennsylvania. Lung cancer risks will also be calculated according to levels of arsenic (a known by-product of zinc smelting, lead, zinc, cadmium, and manganese measured from soil samples obtained by Lehigh University.

The calculation of environmental exposures will involve creating a model to take into account the available pollutant data, wind direction, and topography of the area.

DR. HELLMAN: Are you able to examine the relation between chronic pulmonary disease and lung cancer?

DR. POTTERN: We have checked chronic lung disease history in over 90 percent of the lung cancer patients. So we do have a history of that. We also have checked if the next of kin has said a control had a chronic lung disease in the questionnaire, or it might have been mentioned on the death certificate. We went back and reviewed the control records to check if they really had chronic lung disease. So that is one of the factors we can look at. But that's all we really have.

DR. HELLMAN: I also wonder about the progression of the disease from the point of view of possible immunological interactions.

DR. POTTERN: We don't have that information. Probably we might have, you know, when they were first diagnosed, and, of course, we have that, we could look at that. But that's about all we have on the patients. Since it was a next-of-kin study and working through death certificates, it was too difficult to get the past history.

DR. CARNOW: You mentioned a variety of particulates. I am assuming that you are going to be measuring polycylic aromatic hydrocarbons, or are you only going to measure gross particulates. If you are looking at smelters and steel mills as possible carcinogenic sources, it would seem to me that it would be very useful to do that.

DR. POTTERN: Well, we will only be able to look at the gross.

DR. CARNOW: You will only be able to look at the gross.

DR. POTTERN: Yes.

DR. CARNOW: I think that's very unfortunate since benzene soluble particulate levels, including levels of BAP and other carcinogens is critical in examining relationships between air quality and the incidence of cancer. In the studies carried out by us which appeared in the National Academy of Science document on particulate polycylic aromatic hydrocarbons and lung cancer, we found a relationship between levels of benzene soluble particulate and lung cancer, but did not find any significant relationship when we examined gross particulate.

DR. POTTERN: Oh, we will be able to get some of the other measures probably from the soil data that we have. I don't know.

DR. CARNOW: Well, again, I believe that there are two measurements that are important. In preliminary examinations around South Chicago, we have found a high degree of variability of levels and distribution of PAHs. This may be because of the variation in the kind of process that is being used in various operations.

DR. POTTERN: Yes.

DR. CARNOW: And I think that levels of respirable particulates would also be very important to have, at least making a determination of a percentage of the particulate which is 5 microns or less.

DR. POTTERN: Yes. This was state data that was already obtained. I will look into it in a bit more detail to see if they have it broken down any more.

DR. CARNOW: I think in Allegheny County for example they did and they may have such data in other counties also.

DR. POTTERN: Yes, I will check that.

DR. FRAUMENI: Are there any other questions? Thank you, Linda. The next paper is entitled Mortality and Industrial Hygiene Study of Workers Exposed to Styrene, by James Beaumont, NIOSH; the co-project officer is Michael Crandell.

PROCEEDINGS OF THE
SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Mortality and Industrial Hygiene Study of Workers Exposed to Styrene

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MORTALITY AND INDUSTRIAL HYGIENE STUDY OF WORKERS EXPOSED TO STYRENE: INTERIM REPORT

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James J. Beaumont
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Styrene was first discovered in the nineteenth century, although it was not until the late 1930's that a high purity styrene monomer was manufactured commercially.[1] Today styrene is used extensively in the manufacturing of plastics, synthetic rubber and reinforced plastics. NIOSH estimates that 50,000 workers have potential exposures to styrene. Occupations with potential styrene exposure are shown in table I.[2]

Styrene, also known as vinyl benzene, is an unsaturated, aromatic monomer. It is a colorless liquid with a molecular formula of C_8H_8 and a molecular weight of 104.14. Unlike other aromatic hydrocarbons such as toluene, the xylenes, and ethyl benzene which occur naturally, styrene and its oxide are produced synthetically. The most widely used method of manufacturing styrene is by alkylating benzene with ethylene in the presence of a catalyst to form ethylbenzene. The ethylbenzene is then dehydrogenated to form styrene.[3]

Styrene acts primarily as a narcotic and an irritant of the skin and mucous membranes. It may be absorbed into the bloodstream via all routes, including ingestion, inhalation, or absorption through the skin. In industrial settings, inhalation of vapors and mists and skin contact with the liquid are the most common modes of exposure.[4]

Styrene's odor threshold is about 10 ppm and concentrations greater than 100 ppm are regarded as having a very unpleasant odor. Transient irritations of the eyes, nose, and oral cavity may develop when concentrations exceed 100-200 ppm and coughing may occur around 500 ppm.[5,6]

Styrene is a known neurotoxic and hepatotoxic agent. The neurotoxic effects are well recognized and have been demonstrated in animals, such as rats[7], mice[8], and guinea pigs[7]; and in humans, in both experimental[9,10] and occupational settings.[11,12] Central nervous system (CNS) effects have been observed with both acute and chronic styrene exposures. When rats and guinea pigs were continously exposed to styrene concentrations ranging from 1,300 to 10,000 ppm, the animals exposed to the higher concentrations experienced immediate weakness, loss of equilibrium, convulsions, and death within three hours. When exposed to 1,300 ppm, some rats (but no guinea pigs) survived more than 40 hours of continuous exposure.[7] Humans experimentally exposed to 800 ppm styrene showed immediate CNS effects of listlessness, drowsiness, and impaired balance, all of which continued after exposure[9]. At styrene concentrations of 100 ppm or more, overt CNS effects have been observed in humans exposed for as little as 30 minutes. These effects included inability to perform Romberg tests,[10] decreased manual dexterity and coördination,[10] and increased reaction time.[6,9] Subjective CNS effects such as headache, unusual fatigue, difficulty concentrating, and a feeling of tension were experienced during styrene exposure and persisted after exposure ceased. The magnitude and duration of the signs and symptoms increased with the duration and level of the exposure.[10] Workers exposed to styrene in the manufacturing of reinforced plastics had increased reaction time,[11,12] abnormal electroencephalograms,[13] and subjective symptoms such as headache, fatigue, tension and dizziness.[12,13]

The hepatotoxic effects of styrene have been hypothesized to be due to a metabolite of styrene binding covalently to liver protein in the same way as other known hepatotoxic agents.[14] Rats exposed to a single dose of 1,300 ppm for 30 hours had apparent damage to the liver parenchyma, although at similar exposure levels, no liver damage was found for either rabbits or monkeys.[7] Rats intubated with 400 mg styrene/kg/day, 5 days a week for 24 weeks had decreased liver weights[15] and after a single dose of styrene to rats (2.5 g/kg), a 50% decrease in hepatic glycogen was found.[16] Investigators have reported liver damage in humans exposed to styrene based upon a variety of clinical tests such as increased serum enzymes,[17,18] increased serum gamma-globulin,[19]and elevated serum uric acid levels.[20] Increased GGTP activity was demonstrated in polystyrene workers in Germany[17] and the United States.[18]

The evidence concerning styrene as a carcinogen is unclear. Styrene has shown little and poorly reproducible potential for mutagenicity[21,22] although occupational exposure to humans has indicated that styrene may cause germinal or somatic mutations. Workers who manufactured fiberglass reinforced plastics products showed more frequent abnormal chromosomes in lymphocytes than controls.[23,24] Also, an excess number of lung tumors (adenomas and carcinomas) have been produced in male mice exposed to styrene; however, no excess tumors were produced in female mice or rats of either sex in that same study[25].

Part of the concern over the potential carcinogenicity of styrene is based upon the fact that styrene oxide may be an intermediate in the metabolism of styrene, and that styrene oxide has been shown to be mutagenic to microorganisms.[26-28] The evidence in favor of this hypothesis is that microsomes from livers of rats pretreated with phenobarbital converted styrene to styrene oxide.[29] Based upon this evidence a scheme for the metabolism of styrene to mandelic acid through styrene oxide and phenylglycol was proposed.[29,30] However, when styrene oxide was fed to rabbits, phenylglycol was not found[31] and there have been no investigations which showed the presence of styrene oxide as a product of styrene metabolism in vivo.

Few epidemiologic mortality studies of styrene have been completed to date. Spirtas et al.[32] conducted a case-control study of workers at a rubber manufacturing plant. Their results indicated that working in a synthetic rubber plant may be associated with neoplasms of the lymphatic and hematopoietic tissues as well as a group of lymphomas. Nicholson et al[33] and Frentzel-Beyme et al.[34] reported negative results in mortality studies of styrene-polystyrene polymerization workers. Ott et al.[35] examined the mortality experience of employees in the development or production of styrene-based products. The results revealed that deaths due to malignant neoplasms were fewer than expected for the total cohort, although an increase in lymphatic leukemia was observed among a subgroup of employees who had exposure to polymer extrusion fumes, solvents, and colorants. Werner[36] reported a statistically significant excess of lymphoma deaths among men who worked at least one year in the production, polymerisation and processing of styrene. The author suggested caution when interpreting the results, due to small numbers (3 lymphoma deaths) and lack of association with duration of exposure.

To further investigate the health effects of styrene, NIOSH is conducting a cohort mortality study. The employees from two fiberglass reinforced plastic boat manufacturing facilities in Washington State were selected as the cohort to be utilized. The selection of an appropriate cohort was difficult since the industry is relatively new and the number of years since initiation of exposure for most individuals is relatively short. To support the mortality study, in-depth industrial hygiene surveys were conducted at seven boat manufacturing facilities, two of which are being used in the mortality study. These surveys were used to determine the employees' full-shift time weighted average exposure to styrene. The permissible exposure level for styrene is an eight-hour time-weighted average of 100 ppm, with a five-minute short-term exposure limit of 200 ppm.

The production of reinforced plastic boats begins by preparing a reinforced plastic mold. The mold has the converse shape of the finished part and is coated with a release agent, usually a wax. A layer of gel coat, a pigmented polyester resin, is then applied to the mold surface using an airless sprayer system in a ventilated booth. After the gel coat has set, the hand lay-up or laminating begins. Alternating layers of catalysed resin and fiberglass are applied over the gel coat. Two forms of fiberglass are used: woven roving and chopped strand roving. These are applied starting with the chopped fiberglass, which can be applied using a chopper gun or in a mat form, and followed by the woven roving. Each layer is saturated with a resin containing styrene, using either a spray gun or brush. After the resin is applied, each layer is squeegeed to remove excess resin and rolled out to remove air pockets and other imperfections. Layers continue to be build up until the desired thickness is obtained.

The exposure to styrene results from the use of thermoset polyester resins. The type used in boat manufacturing contains approximately forty percent by weight styrene monomer as a reactive dilutent. During manual laminating and spraying operations, as much as ten percent of the styrene volatilizes into the workplace air with the remainder being consumed in the chemical reaction.

Table II shows the composite mean concentrations of styrene for the seven plants surveyed. Plants A and B are the facilities being used in the cohort mortality study. Their mean concentrations of styrene were 42.5 and 71.7

ppm. The lowest mean concentration among the 7 plants was 35.9 ppm in plant F and the highest mean concentration was 90.0 ppm in plant D.

In addition to varying exposures by plant, there are also factors within each job category which determine the amount of styrene to which a worker would be exposed. These include the size and configuration of the part being made, which effects the amount of resin used and the method of resin application. The size of the part determines the surface area over which the catalyzed resin is applied and, therefore, evaporates. Size is also a factor in the length of time necessary to squeegee and roll out the applied layers. During the rolling operation, the rate of styrene evaporation is the greatest and the workers are closest to the mold surface. An example of the influence of both size and part configuration is in hull lamination. It requires the largest volume of resin and the configuration of the hull is such that styrene vapors may become entrapped. Tables 3 through 5 show the mean styrene concentrations for particular job categories for all plants combined and for Plants A and B separately.

The individuals included in the cohort mortality study are employees from two of the boat manufacturing facilities. Styrene exposure began at both facilities in 1957. At the time of the data collection in October 1978, there had been 1787 employees at plant A and 3581 employees at plant B. All the employment records at both facilities were microfilmed and the demographic data was coded into a computer file.

To assess the completeness of the plant personnel records, the companies were requested to obtain quarterly earnings reports from the Social Security Administration. This information was coded into a computer file and matched against the individuals in the study population. The discrepancies between the two files are being clarified with the help of both companies.

The vital status of each cohort member is being ascertained through the use of the Social Security Administration and Washington State Department of Motor Vehicles. Follow-up through Social Security (through December 31, 1977) yielded 78% with a known vital status. This low percentage is partially due to the inclusion of women in the study and the problem of tracing individuals who may have been known by more than one last name. To help resolve this issue, all individuals with unknown vital status have been resubmitted to the SSA. For the female employees, both most recent name on the personnel records and any former names were included.

The initial follow-up revealed that very few cohort members were deceased (approximately 3%) as of December 31, 1977 (Table VI). The low rate appears to be partially due to the industry being relatively new. For those known to be deceased, 75 percent of the death certificates were located.

The white male portion of the cohort was analyzed by a modified life table technique taking into account the confounding variables of race, sex, age, and calendar year. The external comparison population was United States white males. As seen in Table VII, the only increased cancer was male genital cancer. Of the four deaths in this category, three were prostatic cancer and one was testicular.

For deaths due to the lung cancer or lymphatic and hematopoietic cancer (the cancers of *a priori* interest due to previous reports), statistical power calculations were performed to determine the study's ability to show statistically significant excesses. As shown in Table VIII, the relative risk for lung cancer would have to be approximately 2.0 or greater to have substantial power (at least 80%). For lymphatic and hematopoietic cancers, the relative risk would have to be even larger. These calculations do not take into account the short interval that most workers have had to develop cancer if there is an excess risk.

There will be two main objectives in further analysis: the first objective will be to determine if the employees as a total group have experienced any excess cause-specific mortality risks when compared to the external comparison population. The second objective will be to determine if the employees have experienced any cause-specific mortality risks associated with any specific departments or jobs within the two facilities. To facilitate this objective, employees are being grouped into categories by levels of styrene exposure according to the NIOSH industrial hygiene surveys and recommendations of the two companies.

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TABLE I

POTENTIAL OCCUPATIONAL EXPOSURES

- Adhesive makers
- Boat makers
- Emulsifier agent makers
- Insulator makers
- Organic chemical synthesizers
- Petroleum refinery workers
- Plastic luggage makers
- Polyester resin laminators
- Polystyrene makers
- Potting compound workers
- Protective coating makers
- Rubber makers
- Sports car body makers
- Styrene workers
- Swimming pool makers
- Varnish makers

TABLE II

COMPOSITE MEAN CONCENTRATIONS OF STYRENE FOR THE SEVEN PLANTS SURVEYED

Plant	Number Sampled	Mean (ppm)
*A	53	42.5
*B	67	71.7
C	38	86.4
D	69	90.0
E	62	65.5
F	116	35.9
G	59	82.4
All	464	63.8

*Plants Included in the Cohort Mortality Study

TABLE III
RANGE AND MEAN TWA STYRENE EXPOSURES BY JOB PERFORMED AT ALL SEVEN PLANTS

Job Performed	Number Sampled	Styrene Concentrations (ppm) Range	Mean	Std. Dev.
Hull Lamination	160	1.56 - 183	76.3	40.9
Deck Lamination	114	12.3 - 160	73.4	39.4
Small Parts Lamination	54	9.27 - 130	45.0	21.8
Gel Coat	45	5.30 - 103	47.5	27.4
Large Parts Lamination	41	9.27 - 98.3	43.8	21.6
Stringer Installation	14	33.6 - 156	86.4	42.0
Model Development	7	45.0 - 88.5	64.6	17.9
Mold Work	12	7.34 - 52.6	27.8	16.0
Overlay & Patch	6	10.4 - 61.3	31.1	18.9
Foam & Chop	5	24.1 - 33.5	28.7	3.53
Paste Mixer	1		21.4	
Other	5	15.1 - 77.3	32.3	25.8
Composite	464	1.56 - 183	63.3	

TABLE IV
RANGE AND MEAN TWA STYRENE EXPOSURES BY JOB PERFORMED AT PLANT A

Job Performed	Number Sampled	Styrene Concentrations (ppm) Range	Mean	Std. Dev.
Hull Lamination	18	12.0 - 82.7	51.1	17.8
Deck Lamination	11	12.3 - 66.2	37.2	16.3
Small Parts Lamination	15	20.1 - 84.7	45.8	17.6
Gel Coat	4	22.5 - 44.9	34.2	9.52
Mold Repair & Patch	4	7.34 - 19.1	11.8	5.32
Model Development	1		53.7	
Composite	53	12.0 - 84.7	42.5	19.0

TABLE V
RANGE AND MEAN TWA STYRENE EXPOSURES BY JOB PERFORMED AT PLANT B

Job Performed	Number Sampled	Styrene Concentrations (ppm) Range	Mean	Std. Dev.
Hull Lamination	27	33.6 - 183	96.1	40.8
Deck Lamination	12	31.4 - 58.3	44.7	10.4
Small Parts Lamination	8	23.7 - 46.8	33.7	8.05
Gel Coat	2	28.7 - 29.4	29.0	0.49
Stringer Installation	8	33.6 - 156	106	45.8
Overlay & Patch	6	10.4 - 61.3	31.1	18.9
Model Development	4	63.1 - 88.5	76.6	13.6
Composite	67	10.4 - 183	71.7	42.9

Table VI

STYRENE
FOLLOW-UP AS OF DECEMBER 31, 1977

Vital Status	Number	Percent
Alive	4024	75
Deceased	182	3
Unknown	1162	22
Total	5368	100

Table VII

PRELIMINARY MORTALITY RESULTS

Death Category	Observed	Expected
Lung Cancer	11	10.1
Lymphatic and Hematopoietic Cancer	0	3.9
Male Genital Cancer	4	1.7
Other Cancer	13	15.0
All Deaths	156	172.5

Table VIII

Styrene
Power to Detect Excess Risk

Death Category	RR = 1.5	RR = 2.0	RR = 3.0
Lung Cancer	41%	84%	99%
Lymphatic and Hematopoietic Cancer	22%	49%	89%

DR. FRAUMENI: Dr. Beaumont, are there confounding environmental exposures of concern, like asbestos?

DR. BEAUMONT: Fiberglass is the only other exposure of substance at all. There are no known health effects associated with that. But again, that is a relatively new substance and the story isn't complete there yet.

MR. SCHNEIDER: Scott Schneider, United Brotherhood of Carpenters. I had a question about styrene oxide exposures because I know in some of the Scandinavian literature there have been some suggestions that people who are exposed to styrene are also being exposed to styrene oxide. Has that been checked at all? Has that been sampled for?

DR. BEAUMONT: We didn't sample for it and I haven't seen that literature in particular. This is the first I've heard that might be what exists.

MR. KENT: The preliminary calculations that you made, where did you draw out your expected numbers? Were they for the whole cohort or just the number that you had a successful follow-up on?

DR. BEAUMONT: They are for all of the white males. We included the unknowns also. We considered them alive to the end of the study.

SPEAKER: Do you have any plans to find those persons and if you haven't considered it as yet, I would encourage you to because they will take your cohort as a test, and since all of the mortality isn't being reported from January 1, 1979, it won't cost you anything and you can turn it around fairly quickly.

DR. BEAUMONT: Right now, Social Security, which is our primary source of follow-up is only telling us about vital statistics through 1978. Then this new system starts in 1979?

SPEAKER: January 1, 1979 and they are taking test cases right now to test out the matching number. So I would encourage you to take the information you have on this cohort.

DR. BEAUMONT: Yes, it sounds like we could do that, yes. Thank you.

DR. FRAUMENI: Could I ask about the previous reports of leukemia following styrene exposures? Do these seem to have short latent periods so that if there is a risk, you might be able to pick up some cases on a study of this nature?

DR. BEAUMONT: Frankly, I haven't looked at those studies that closely. Leukemia typically has a shorter latency period than other cancers. I think this has been shown both with radiation and with benzene. In the synthetic rubber industry I think they were shorter, maybe in the 10 to 20 year range. That's all I know at this point.

PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Preliminary Report on A Case-Control Study of Cancers of the Lung, Stomach, and Pancreas in Southern Louisiana

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PRELIMINARY REPORT ON A CASE-CONTROL STUDY OF CANCERS OF THE LUNG, STOMACH, AND PANCREAS IN SOUTHERN LOUISIANA

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INTRODUCTION

With the publication of the Atlases of Cancer Mortality for U.S. Counties: 1950-1969, it became apparent that cancer mortality rates in southern Louisiana are significantly higher than rates for the United States as a whole for all sites combined and for particular site/sex/race groupings (1,2). Studies to determine factors associated with excess risk for cancer of the lung in Louisiana utilizing death certificate analysis reported a two-fold excess lung cancer risk associated with certain industries in 19 south Louisiana parishes (3). In order to further investigate the impact of potential risk factors on cancers of the lung, stomach, and pancreas, a case-control study of these cancers diagnosed among residents of south Louisiana was undertaken. The purpose of this research is to evaluate environmental determinants of each of these cancers in south Louisiana by a comparison of the characteristics and interview responses of cancer cases and their matched controls.

METHODS

Cases of primary lung cancer, primary stomach cancer, and primary pancreas cancer diagnosed in residents of 26 south Louisiana parishes * have been identified since 1979. The 26 parishes included in the study extend from the Texas-Louisiana border to the Mississippi-Louisiana line and from the Gulf of Mexico to the middle of the state in a north-south direction. Twenty-five hospitals are participating in the study. Outside the metropolitan

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*The parishes include Acadia, Ascension, Assumption, Avoyelles, Calcasieu, Cameron, East Baton Rouge, Evangeline, Iberia, Iberville, Jefferson, Jefferson Davis, Lafayette, Lafourche, Orleans, Pointe Coupee, St. Bernard, St. Charles, St. James, St. John the Baptist, St. Landry, St. Martin, St. Mary, Terrebonne, Vermillion and West Baton Rouge.

New Orleans area, nearly total coverage exists. Within the metropolitan area, a sample of public and private hospitals are included.

Controls are selected from hospital admissions and are matched to cases by hospital, race, sex, and age (± five years) and must also be residents of the twenty six study parishes. Certain exclusions for controls of each cancer site were made. Admission exclusions for controls matched to lung cancer cases are chronic lung disease (emphysema, chronic bronchitis, and obstructive pulmonary disease) and cancers of the larynx, pharynx, oral cavity, esophagus, and urinary bladder. Admissions for ulcers of the stomach or duodenum are excluded as controls for stomach cancer cases, and admissions for cirrhosis of the liver and cancer of the abdominal cavity NOS are excluded as controls for pancreas cancer cases.

Personal interviews of the patients and controls, or their next of kin in the event that they have died, are conducted by locally hired interviewers. Each interviewer has been trained in interviewing techniques and in the use of the questionnaire. Most questions are in closed form to minimize inter-observer biases. Sample questions in 5% of all interviews are later asked for verification by telephone.

Information is obtained on place, type, and length of employment for all jobs held six months or more. Respondents are asked to describe the duties performed for each job, as well as to list materials handled during the period of employment. Information on leisure time activities is also obtained with regard to type of activity and any materials involved. This section of the interview is concluded by a review with each respondent of a checklist of industries and materials. Information on lifetime residential history is obtained for residences of six months or more. The type of water supply at each residence is also asked of each respondent. A detailed smoking history is obtained and includes questions on all forms of tobacco use. Initiation, cessation, duration, type (filter, non-filter, rolled), and brand are determined for persons who report ever having smoked cigarettes. The diet section is composed of questions concerning the frequency of consumption of certain food items, use of certain spices, and consumption of beverages including alcoholic beverages by type and brand, soft drinks, tea, and coffee by type and brand. Information is also collected on country of origin and Acadian ancestry/culture.

Preliminary analysis has included estimates of relative risk (odds ratios) for various smoking, occupation, and dietary categories (4). Summary relative risks have been determined by the Mantel-Haenszel method (5).

RESULTS

A total of 619 cases of primary lung cancer and 536 controls are included in this analysis. A total of 170 cases of stomach cancer and 153 controls, and 124 cases of pancreas cancer and 123 controls are also included in this analysis. These cases and controls represent only a portion of the final study group and the results reported should be interpreted only as preliminary.

A total of 1842 lung cases and 1434 controls have been identified as of August, 1981. Interviews have been completed for 70% of the lung cases and 86% of the controls. A total of 229 pancreas cancer cases and 174 controls have been identified with interviews completed for 76% of these cases and 91% of the controls. A total of 319 stomach cancer cases and 232 controls have been identified, and 73% of the cases and 92% of the controls have been successfully interviewed. Interviewing of lung cancer cases was completed on June 30, 1981; however, identification and interviewing of stomach and pancreas cancer cases will continue into 1982.

The major findings in this preliminary analysis pertain to smoking history and lung cancer. As shown in Table 1, the largest differences in tobacco use between lung cancer cases and controls are for the categories "no tobacco use", 3.2% of the cases versus 22.8% of the controls, and "cigarette only", 70.3% of the cases versus 47.6% of the controls.

Lifetime maximum daily cigarette consumption among smokers was then determined for cases and controls and a statistically significant test for trend was found as shown in Table 2. Approximately 88% of the lung cancer cases who reported ever having smoked did so at a level of at least one pack per day compared to 74% of the controls, and twice the proportion of cases as controls ever smoked three or more packs per day.

Table 3 shows the type of cigarettes smoked by cases and controls. While the proportion of nonsmokers among cases and controls dramatically differ, among the smokers both cases and controls reported similar use of filter cigarettes, nonfilter cigarettes, and the combination. An examination of the ages of respondents who reported switching from nonfilter to filter cigarette use revealed that nearly twice the proportion of controls compared to cases made the transition below the age of thirty. (Table 4)

A relative risk estimate of 1.54 (1.18,2.0) was found for initiation of cigarette usage below the age of sixteen years as shown in Table 5.

Information obtained on smoking cessation from respondents revealed that 77.4% of cases and 66.5% of controls who ever smoked are current smokers. As shown in Table 6, among those smokers who are not current cigarette smokers, nearly twice the proportion of controls as cases stopped smoking more than ten years before diagnosis, and the proportion of controls was double that of cases who stopped smoking more than 20 years before diagnosis.

In an attempt to compare lifetime pack-year histories of cases and controls and account for risk differences in filter and nonfilter cigarette use, an adjusted pack-year history was determined, giving a weight of 0.75 to filter pack-years and 1.0 for nonfilter pack-years and summing over lifetime use (Table 7). Again large differences are seen, and the estimates of relative risk double for each higher category of pack-year consumption. Differences in relative risk were found when adjusted pack-year histories of cases and controls were examined within socioeconomic categories. Respondents were classified by socioeconomic status (SES) according to the type of hospital to which each was admitted, public or private. As can be seen in Table 8, for persons with a pack-year history of less than or equal to 20 and those with a pack-year history of 21-50, the risk in the low socioeconomic category was double the risk found in the high SES category. For the highest level of cigarette consumption, over 50 pack-years, relative risks of almost 20 were found in both levels of SES.

Preliminary analysis of the stomach cancer data has been limited to an examination of vitamin intake. An index of vitamin C intake was constructed by summation of the monthly frequency of consumption of foods containing at least 22 milligrams of vitamin C per 100 gram serving (Table 9). Similarly, an index of vitamin A intake was constructed by summation of the monthly frequency of consumption of foods containing at least 500 International Units of vitamin A per 100 gram serving (Table 10). Significant differences in consumption patterns of stomach cancer cases and controls were found for both indices. Lower intake levels of vitamin A- and C-containing foods were found for stomach cancer cases than for controls.

For cancer of the pancreas, preliminary analysis has been limited to an examination of coffee consumption. Respondents were asked about current coffee drinking habits, and the results are shown in Table 11. Decreased risk was found for light and moderate daily coffee consumption of one to four cups per day, and a slightly (non-significant) elevated risk, R.O. 1.3, for daily consumption of five or more cups.

DISCUSSION

The results presented in the preceding text and tables represent a very basic, preliminary analysis of an incomplete data set. The most appropriate discussion should focus on plans for further analysis of the data.

For cancer of the lung, our preliminary analysis has concentrated on the role of smoking and, as expected, it was found to be highly, significantly associated with lung cancer. Our preliminary analysis of the smoking histories of study subjects has shown that controls report switching from nonfilter to filter cigarettes at an earlier age than cases and that there is an elevated relative risk associated with initiation of cigarette usage below the age of sixteen. The role of smoking habits during young adult life will be further examined. Additional analysis of the smoking variables will include an examination of both maximum amount smoked and current amount smoked adjusting for age at diagnosis, socioeconomic status and starting age. Relative risks for pure cigarette users will be compared to combined tobacco users, and all risks will be calculated using "never used any tobacco" as the baseline. The key smoking variables will ultimately be included in some type of model of lung cancer risk derived from logistic analysis of the data.

A major set of variables yet to be analyzed includes those of the industry and occupational section of the questionnaire. Ever employed in an industry and industry of usual employment are two basic approaches to analysis, both of which will be utilized. Specific occupations and occupationally-related exposures to certain materials will also be analyzed together with information about industry. Socioeconomic status, age at diagnosis, race, and smoking history are all potentially confounding variables which must be accounted for in the analysis.

Consumption of certain items of food such as cabbage, lettuce and milk which have been positively or negatively associated with stomach cancer in other studies (7,8,9), as well as frequency of consumption of broad categories of food and indices of vitamin intake, will be included in the complete analysis of factors associated with excess risk for stomach cancer. The role of tobacco use, alcohol consumption, age, race, and socioeconomic status,alone and in combination with the food items,must also be considered.

Analysis of the data gathered on cases of pancreatic carcinoma and controls is of particular interest in light of recent reports suggesting a possible association with coffee consumption (10, 11). Information has been asked of respondents on current daily frequency of coffee consumption, usual brand, caffeine content, and chicory content. Our preliminary calculation of relative risk estimates within categories of coffee consumption does not show a straightforward dose response effect, with decreased risk in the light and moderate coffee consumption categories and a slightly elevated risk in the heavy coffee drinking category. Further analysis of this variable will include examination of odds ratios within age, sex, and race categories, and the possible association of coffee consumption with pancreatic cancer will be examined in combination with and with adjustment for alcohol consumption and tobacco use.

With at least 1250 case-control pairs, there should be sufficient study subjects in the lung cancer portion of the study to stratify on a number of variables simultaneously and still meet minimum cell size requirements prior to developing a model. For cancers of the stomach and pancreas, there will be approximately 250 case-control pairs in each data set. Stratification and use of the Mantel-Haenszel test statistic will be useful for consideration of two or three variables simultaneously; beyond that, logistic analysis will be used.

TABLE 1

TYPE OF TOBACCO USAGE AMONG LUNG CANCER CASES AND CONTROLS IN SOUTHERN LOUISIANA

	NO. OF CASES (%)	NO. OF CONTROLS (%)
NO TOBACCO USE	20 (3.2)	122 (22.8)
COMBINED USE	159 (25.7)	124 (23.1)
CIGARETTE ONLY	435 (70.3)	255 (47.6)
USE OTHER THAN CIGARETTES	5 (0.8)	35 (6.5)
TOTAL	619	536

TABLE 2

LIFETIME MAXIMUM DAILY CIGARETTE CONSUMPTION AMONG SMOKERS IN SOUTHERN LOUISIANA BY LUNG CASE/CONTROL STATUS

CIG./DAY	NO. OF CASES (%)	NO. OF CONTROLS (%)
1- 9	16 (2.7)	38 (10.2)
10-19	58 (9.8)	60 (16.0)
20-39	270 (45.5)	179 (47.9)
40-59	164 (27.7)	69 (18.4)
60+	85 (14.3)	28 (7.5)
TOTAL	593 (100)	374 (100)

$x^2 = 47.779$

$p = 0.0001$

TABLE 3

TYPE OF CIGARETTE USE AMONG LUNG CANCER CASES AND CONTROLS IN SOUTHERN LOUISIANA

	NO. OF CASES	NO. OF CONTROLS
NONSMOKERS	25	157
BOTH FILTER AND NONFILTER	381	231
FILTER ONLY	25	23
NONFILTER ONLY	188	125
TOTAL	619	536

TABLE 4

AGE AT TRANSITION FROM NONFILTER TO FILTER CIGARETTE USE AMONG LUNG CASES AND CONTROLS WHO SWITCHED TYPE

	NO. OF CASES (%)	NO. OF CONTROLS (%)
10-19 YRS OLD	7 (2.0)	6 (2.9)
20-29 YRS OLD	37 (10.5)	40 (19.2)
30-39 YRS OLD	109 (30.9)	55 (26.4)
40-49 YRS OLD	100 (28.3)	69 (33.2)
50-59 YRS OLD	70 (19.8)	27 (13.0)
60 & OVER	30 (8.5)	11 (5.3)
TOTAL	353	208

TABLE 5

ESTIMATE OF RELATIVE RISK OF LUNG CANCER ASSOCIATED WITH INITIATION OF CIGARETTE USAGE AT AN EARLY AGE

	Lung Cancer	
	Case	Control
< 16 years of age	281	139
16 + years of age	312	237
TOTAL	593	376

R.R. = 1.54

(1.18, 2.0)

TABLE 6

SMOKING CESSATION AMONG LUNG CANCER CASES AND CONTROLS IN SOUTHERN LOUISIANA

	NO. OF CASES (%)	NO. OF CONTROLS (%)
NON-SMOKERS	25	157
CURRENT SMOKERS	460 (77.4)	252 (66.5)
QUIT 3-5 YEARS	36 (6.1)	29 (7.6)
QUIT 6-10 YEARS	33 (5.6)	19 (5.0)
QUIT 11-20 YEARS	39 (6.6)	37 (9.8)
QUIT 20+ YEARS	26 (4.4)	42 (11.1)

$x^2 = 22.705$

$p = 0.0001$

TABLE 7

PACK-YEAR HISTORY ADJUSTED FOR FILTER USAGE AMONG LUNG CANCER CASES AND CONTROLS IN SOUTHERN LOUISIANA

	NO. OF CASES (%)	NO. OF CONTROLS (%)	CRUDE R.O.
NONSMOKERS	25	157	1.0
SMOKER -D.K.AMT	1 (.2)	5 (1.3)	-
≤ 20 PK-YRS	72 (12.1)	112 (29.6)	4.04
21-50 PK-YRS	268 (45.1)	174 (45.9)	9.67
51+ PK-YRS	253 (42.6)	88 (23.3)	18.05

$x^2 = 66.953$

$p = 0.0001$

TABLE 8

RELATIVE RISKS OF LUNG CANCER ASSOCIATED WITH SOCIOECONOMIC STATUS AND WITH PACK-YEAR HISTORY OF CIGARETTE CONSUMPTION

	LOW SES	HIGH SES	ADJUSTED FOR SES
NONSMOKERS	1.0	1.0	1.0
≤ 20 PK-YR	6.375	3.478	4.06
21-50 PK-YR	15.576	8.215	9.69
51+ PK-YR	19.91	19.70	19.74
ADJUSTED FOR SMOKING	13.95	10.46	

TABLE 9

INDEX OF VITAMIN C CONSUMPTION AMONG STOMACH CANCER CASES AND CONTROLS IN SOUTHERN LOUISIANA

FREQUENCY OF CONSUMPTION	NO. OF CASES (%)	NO. OF CONTROLS (%)
0/MONTH	1 (.6)	4 (2.6)
1-25/MONTH	58 (34.1)	22 (14.4)
26-50/MONTH	60 (35.3	56 (36.6)
51-75/MONTH	39 (22.9)	46 (30.1)
75+/MONTH	12 (7.1)	25 (16.3)
TOTAL	170 (100)	153 (100)

$x^2 = 22.45$

$p = 0.0002$

TABLE 10

INDEX OF VITAMIN A CONSUMPTION AMONG STOMACH CANCER CASES AND CONTROLS IN SOUTHERN LOUISIANA

FREQUENCY OF CONSUMPTION	NO. OF CASES (%)	NO. OF CONTROLS (%)
1-25/MONTH	12 (7.1)	6 (3.9)
26-50/MONTH	28 (16.5)	15 (9.8)
51-75/MONTH	45 (26.5)	31 (20.3)
75+/MONTH	85 (50.0)	101 (66.0)
TOTAL	170 (100)	153 (100)

$x^2 = 9.016$

$p = .029$

TABLE 11

COFFEE CONSUMPTION AMONG PANCREAS CANCER CASES AND CONTROLS IN SOUTHERN LOUISIANA

COFFEE CONSUMPTION	NO. OF CASES (%)	NO. OF CONTROLS (%)	R.O.
NON-DRINKER	23 (19.7)	19 (15.4)	1.0
LIGHT DRINKER (1-2 CUPS/DAY)	40 (34.2)	56 (45.5)	0.59
MODERATE DRINKER (3-4 CUPS/DAY)	21 (17.9)	27 (21.9)	0.64
HEAVY DRINKER (5+ CUPS/DAY)	33 (28.2)	21 (17.1)	1.30

SPEAKER: Did you gather data on the drinking water?

DR. CORREA: Yes, we have that in the questions.

DR. BLOT: The slide you presented on occupation indicated that about three to five percent of the men in the southern part of Louisiana were employed in the shipbuilding industry. Is that a current figure?

DR. CORREA: Yes, this is a current figure published by the State of Louisiana about two years ago.

DR. BLOT: Do you have an idea of what percent of the population was employed in shipbuilding during World War II?

DR. CORREA: No, we don't have a good idea. We know it was much greater than now because we have a life-long occupational history taken at the interview and we have a lot of people telling us that they were working in that industry during the war.

DR. BLOT: Have you been able to look at case control differences?

DR. CORREA: No, we have not yet.

DR. FRAUMENI: And you have a cluster of mesothelioma in southern Louisiana?

DR. CORREA: We have a group of cases. I don't know if it is higher in the south than in the north. We are looking at them right now but preliminary analysis seems to indicate that there is a cluster.

PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Mortality Study of Workers in the Plywood, Paper and Pulp Industries

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Approximately 600,000 people are currently employed in the paper industry (1) and 180,000 people in the plywood industry (2) in the United States. These industries involve occupational exposures to sulfuric acid, chlorine, formaldehyde, pigments, and various other potentially toxic chemicals in addition to wood dust. Suspicions that employees in these and other wood related industries might be at increased risks of malignancies were raised initially in 1965 by Macbeth (3) who found excessive numbers of nasal cancers among chair-makers. Since then numerous epidemiologic studies have found associations between work in a variety of wood related industries and the subsequent occurrence of site specific malignancies (Table 1). Workers in the paper pulp and plywood industries have been observed to be at increased risk for some of these malignancies.

A study conducted by Milham (1) found excess proportionate risks of death due to coronary heart disease associated with employment in paper pulp mills and plywood mills. Additionally he found paper pulp mill workers had a statistically significant increased proportion of deaths due to Hodgkin's Disease and nonstatistically significant elevated proportions of deaths due to cancer of the small intestine, lymphosarcoma, multiple myeloma, lymphatic and monocytic leukemia and nonmalignant diseases of the blood and blood-forming organs. Plywood mill workers were observed to have statistically nonsignificant increased proportions of deaths due to cancer of the stomach, multiple myeloma, myeloid and acute leukemia.

Respiratory cancer was slightly elevated for deaths occurring during 1950-1960 only.

Two other studies have linked employment in the paper industry with lymphatic malignancies. In a case/control study by Milham and Hesser (2) (1967), a 4 fold risk for Hodgkin's disease was found among paper mill workers. A case control study, reported by Decoufle (3), et al (1977) and Bross (4) (1978), found that men who had worked as operatives in the paper industry for 5 years or more had a relative risk of 2.4 for lymphomas, in addition to a relative risk of 3.2 for leukemia.

Lung cancer was associated with pulp and paper mill employment in three out of five studies. Blot and Fraumeni (5) (1976) reported that the age-adjusted annual lung cancer mortality rates were significantly higher among white males of the Eastern and Southern but not Western counties where pulp and paper mill industries were located. Menck and Henderson (6) (1976) calculated a statistically significant risk ratio of 1.7 for observed deaths and incident cases of lung cancer among white males whose last known industry in which they had been employed was paper manufacturing and sales. Gottlieb (9) (1979) reported finding no excess risk of lung cancer among those employed in the paper industry in a review of lung cancer deaths which occurred between 1960 and 1975 in Louisiana. No smoking information was available for any of thse studies (5,6,9). An increased relative risk of 1.28 for deaths due to lung cancer was found by

Harrington (7) (1978) among workers in wood and paper industries located in rural but not in urban areas. However, in a followup study of the same geographic region, Blot (8) (1978) reported no increased relative risk for lung cancer associated with employment in paper mills after adjusting for cigarette smoking.

A four-fold risk for oral and laryngeal cancer was observed by DeCoufle (3) (1977) and Bross (4) (1978) among men who had worked as operatives in the paper industry for 5 years or more. A significantly increased mortality rate for oral cancer was observed among white males by Blot and Fraumeni (1977) for U.S. counties with more than one percent of the population employed in the paper, leather, and chemical manufacturing industries.

Nasal cancer, but not Hodgkin's disease, has been shown to occur in great excess among workers exposed to hard wood dust during their employment in the furniture industry (Acheson (15) 1967, Milham and Hesser (16), (1967) and Brinton (17) et. al., (1976)). Finally, soft-tissue sarcomas and histiocytic lymphomas have been reported in three Swedish studies (Eriksson (12) et. al. 1979 Hardell (13), 1979, Hardell and Sandstrom (14), 1979) following exposure to chlorophenols, a potential exposure from wood preservatives among lumber and saw mill workers and others who work with logs and raw woods.

In the only retrospective cohort mortality study reported in the literature Ferris (10) (1979) followed a small cohort of 271 United States pulp and paper mill workers identified as actively working in a mill in 1963. Although only 33 deaths occurred among these workers, the 16 deceased paper mill workers were reported to have an overall SMR of 162. No cause-specific SMR's were computed, but despite the short latency and lack of statistical power, he observed one lymphoma, one kidney cancer, 2 stomach/peritoneal cancers, and 3 lung cancers. All but kidney cancer have been reported elsewhere to be in excess among workers in wood-related industries.

Because of these extensive associations between various malignancies and paper and plywood mill employment, we conducted a retrospective cohort mortality study of men employed in paper pulp mills and plywood mills located in the Northwestern United States. Our study was designed to evaluate the chronic health effects of employment in these two industries, and to test a priori hypotheses of increased mortality due to coronary heart disease, malignancies of the hematopoietic and lymphatic system and malignancies of the mouth, stomach, small intestine, larynx, nose, lung, and soft tissues.

Methods

After an extensive survey of Washington, Oregon and California mills. four plywood mills and five paper pulp mills were selected for study on the basis of the age of the mill, the age and completeness of personnel records and the type of process used by the mill. Table 2 shows that of the five paper pulp mills selected, three produce kraft or sulfate pulp (which are essentially the same), one produces sulfite pulp, and one uses both the calcium-based sulfite and groundwood processes to produce pulp (Table 2).

Because environmentally induced chronic disease can take from 20-50 years before becoming clinically observable, the study cohort was defined so as to exclude workers hired after 12/31/55. In an attempt to facilitate the tracing and follow-up process, only persons who worked at least one year between 1/1/45 and 12/31/55 were selected for study. Employment records of these men were microfilmed at the mills investigated. Their detailed work histories, including departments, job titles and dates of specific jobs held by each man, were computerized using the 21 exposure categories shown in Tables 3 and 4. These categories will be confirmed by industrial hygiene surveys of the mills.

We are following the approximately 3600 paper pulp workers and 2200 plywood workers who met the study definition from their last date of employment through March 31, 1977. Vital status of the cohort members is being determined through records maintained by various government agencies, directories, and other sources. We will analyze mortality patterns separately for the paper pulp mill workers and the plywood mill workers by industry, process and the exposure categories in Tables 3 and 4.

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TABLE 1
PREVIOUSLY REPORTED ASSOCIATIONS BETWEEN WOOD-RELATED INDUSTRIES AND SITE SPECIFIC CANCER

	INDUSTRY			
Anatomic Site of Malignancy	Pulp and Paper	Furniture, Plywood	Sawmills, Lumber Mills and Logging	Carpenters
Buccal and Pharynx	+			
Stomach		+	+	
Small Intestine	+			
Nasal		+	+	
Larynx	+		+	
Lung	+	+		+
Bladder				+
Lymphoma	+			
Hodgkin's Disease	+	+	+	+
Leukemia	+	+	+	+
Soft-tissue sarcoma, histiocytic Lymphomas			+	

+ relative risk greater than 120 unless statistically significant

TABLE 2

STUDY MILLS BY PROCESS AND LOCATION

MILL NO.	INDUSTRY	PROCESS	LOCATION
1	Plywood	Veneer peel	McCleary, WA
2	Plywood	Veneer peel	Shelton, WA
3	Plywood	Veneer peel (limited pressed hardwood chips)	Lebanon, WA
4	Plywood	Veneer peel	Olympia, WA
5	Pulp	Kraft	Tacoma, WA
6	Pulp	Batch sulfate process	Port Townsend, WA
7	Pulp	Sulfate box board	Longview, WA
9	Pulp	Calcium based sulfite 1930-1969 Ammonia based sulfite, 1970 to present	Port Angeles, WA
10	Pulp	Stone groundwood 1920-1964 & calcium sulfite Refinery groundwood 1964-1977	Port Angeles, WA

TABLE 3

PAPER PULP MILL EXPOSURE CATEGORIES

Exposure Category	Exposure Category	Potential Exposures During Study Period
1	Raw wood preparation, i.e. debarking and chipping	wood volatiles, wood dust, (Douglas fir, hemlock), Spores and fungi
2	Sulfate: production of cooking liquor	ammonia, hydrogen sulfide, sulfur dioxide, mercaptans, chromates (as contaminants)
3	Sulfate: pulp production, washing and recovery	lime, magnesium
4	Sulfite: production of cooking liquor	sulfur, sulfur dioxide, calcium carbonate, zinc sulfuric acid, lead fumes, asbestos sulphurous acid
5	Sulfite: pulp production, washing and recovery	pigments, dyes
7	Groundwood: pulp producton, in plant 10	
8	Pulp bleaching and bleach plant	chlorine, neoabietic acids
9	Wet pulp paper additives	talc, clays, titanium dioxide formaldehyde, pigments and dyes
10	Bleaching and pulp additives together	same as exposure 8 and 9 together
11	Paper rolling, sizing, dying drying, glazing, coating	formaldehyde, paper dust, coating and pigment dusts
12	Maintenance	general plant exposures
13	Power and general utility a few other or unknown jobs	general plant exposures
14	Unexposed jobs	

Table 4

Plywood Mill Exposure Categories

Exposure Category	Exposure Category	Potential Exposures During Study Period
1	Raw wood preparation and veneer cutting	wood volatiles, wood dust
2	Pressing and Drying of veneer	neoabietic and other wood acids, volatiles
3	Glue mixing and panel glueing of veneer	pentachlorophenol, carbon tetrachloride, carbon disulfide, flake caustic, lime, sodium silicate, phenol formaldehyde
4	Panel patching of flaws knotholes, etc.	resorcinol formaldehyde resins, ammonium chloride
5	Panel finishing, trimming, sanding	wood dust (mainly Douglas fir, cedar, some hardwoods) wood volatiles, neoabietic and other acids
6	Unexposed jobs	
7	General utility, power maintenance, and a few unknown jobs	general plant exposures

MR. SCHNEIDER: United Brotherhood of Carpenters. Are you going to be looking specifically for formaldehyde exposures in the plywood industry, using those exposure categories?

MS. ROBINSON: The exposure categories are based on information obtained from walk-around surveys and conversations with plant personnel. We are planning industrial hygiene surveys to validate the categories, and formaldahyde is one substance for which we would be looking.

MR. FESTA: American Paper Institute. Cindy, do you think the cohort that you have is large enough to pick up some of the rare lymphatic cancers -- you mentioned Hodgkins disease -- if they exist?

MS. ROBINSON: I don't have any power calculations with me.

DR. FRAUMENI: Are there any other questions? Comments? Are there any other studies? This is a very interesting area, a very important area. Does anybody have any other studies under way that would help clarify the issues involved here. Dr. Kraybill, do you have any instructions for us as to what we do next.

DR. KRAYBILL: My point is with the quality of slides presented here. Please, I hate to be nasty but if you go to other meetings they will not let you present slides with small print. So whoever you are, you ought to print up separate slides, not use the tables or figures that you have for your paper. It's an insult to the audience to show slides and have to apologize because they cannot be read in the back of the room. So you ought to make the print bigger. That is for next year.

PROCEEDINGS OF THE
SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

Thursday Morning, September 10

DISCUSSION GROUP

GROUP A - EPIDEMIOLOGY

Discussion Leader:
Dr. Thomas J. Mason
National Cancer Institute

DR. MASON: Dr. Kraybill has decided that we are going to run a half an hour late, so for approximately the next 30 minutes we will discuss the future direction of these collaborative projects, programs. I am Tom Mason from the NCI, and I have been able to utilize both of the collaborative projects or programs for a number of the studies that we are doing. I would like to ask each of you to comment on the utility of these programs, possible modifications to the approaches, as well as criticisms based on what you have heard to date. You will have an opportunity to do this at least four times today and tomorrow, such that by the last session tomorrow afternoon the decision makers will have available to them some constructive criticism with regard to these projects which will give some indication concerning the direction you believe that these projects or these programs should take.

Honestly, the collaborative programs have not worked most effectively to date. I am of the opinion, and I would encourage discussion if there are other opinions -- that in the early stages these collaborative programs suffered from some personality conflicts within the decision making circles. And I believe that those conflicts have been appropriately resolved.

Now, what I would like to ask of you is this: Against the framework of emerging public health problems, recognizing that these collaborative projects have the potential for providing funding for truly multidisciplinary approaches to population-based epidemiology, I would like to hear from some of the persons who have used the programs and were frustrated by the mechanism and/or pleased, as well as persons who might contemplate utilizing the program.

What modifications, changes, do you think might be in order? Do you believe that there are, and this is an appropriate forum for it; do you believe that there are studies which currently are reasonably well developed from the standpoint of protocols and procedures that are currently lacking funding, that should be considered? Who would be the appropriate persons to basically bring them to the Boards to have them reviewed?

So with that -- Don Austin has probably had the greatest share of problems with regard to using this, so if you could -- Dr. Don Austin from the California State Department of Health -- if you could share, Don, because I think yours is sort of a prototype of the types of studies that have attempted to use this funding mechanism -- I just really need criticisms, comments, with regard to the programs, such that, by the end of tomorrow Dr. Adamson and others will have available to them, hopefully, some input.

DR. AUSTIN: I would be happy to share some of my frustrations with you.

DR. MASON: Good.

DR. AUSTIN: In my area of California we operate one of the local SEER Program areas. This is a population-based cancer reporting system. And in about 1975 we realized that if we had a list of all people of a certain occupation who resided in that occupation-based cancer reporting system we could determine the incidence of cancer in that occupation group.

And if we had also the birth dates of the people on that list we could also determine the expected for the incidence within that group at a very simple cost of just matching one list against another list, i.e., the list of the cancer registry and a list of the people in the occupation.

We started building this concept throught the SEER Program of the National Cancer Institute. About 1976 it was determined that this effort was more in the province of another institute. Subsequently, funding for this activity was cut off, but we were encouraged to apply to the National Institute for Occupational Safety and Health for the same sort of a project. So we went through the business of sending in a proposal. However, the result was an RFP, open to any number of areas. It was also changed from what we wanted to do, and was designed as a feasibility study.

Fortunately, through that feasibility study and working with an outside group we were able to get together about six or seven cohorts of people. A few groups we knew were likely to show a high risk, such as asbestos workers. There were only 235 members in this asbestos workers local. Some groups we felt probably were not at high risk; and some we didn't know about. We matched this against six incidence years of the registry, and sure enough, we identified a significantly high risk for lung cancer and mesothelioma in the asbestos workers. In some of the other groups we showed no elevated risks, the ones that we expected.

We identified a high risk for melanoma in a nuclear research facility cohort in the University of California. Well, now the feasibility study was done. For a portion of the time we got a little bit of funding from OSHA. They thought that this might eventually lead to work place investigations and I am sure that it might eventually do that, but certainly not within the first year of operations.

This program is still almost entirely unfunded. As far as I am concerned, this is a program that really should be developed somewhat on a national basis. It is not a study so much as it is a system, a program, that can break the epidemiology of occupational or environmental cancer into two steps: One, identifying the high risk group and then, secondly, focusing the resources that we have for investigating those identified as high risk groups.

This is a very efficient mechanism. The cancer reporting mechanism is already established. This approach avoids the necessity of setting up a follow-up system for every cohort to be studied. Presently, somewhere between 10 and 20 percent of the population of the United States is covered with population-based cancer reporting systems. It seems like a natural for NIOSH and NCI or EPA and NCI to establish cohorts and follow them through this mechanism, so that we can find out, number one, if they are at high risk and then, focus our somewhat limited resources for case control studies and other investigative field investigations on those that are a high risk.

DR. MASON: Thank you. This is an open discussion so anyone who would like to comment on what Dr. Austin has said, or to take a totally different attack -- Jay, you want to comment?

DR. BEAUMONT: Yes.

DR. MASON: Jay Beaumont from NIOSH.

DR. BEAUMONT: I am a little curious what the resistence has been, why Dr. Austin has had so much trouble? I am wondering if there are technical problems, like what does he propose to do about people who have left the reporting area? How is that handled? Is he talking about a cohort approach or case control approach?

DR. AUSTIN: Well, I am not sure what the resistance has been, except usually people say, well, it's not in our province. It's somebody else's responsibility.

The questions that usually do come up are those that you raised. One is the question of how to establish how much exposure these people had when all that is available are their names and the fact that they worked in an industry or worked in a certain trade?

My answer to that is, we don't know exactly how much exposure they had, except we know that asbestos workers have more exposure to asbestos than other people. People who work in oil refineries have more exposures to petroleum products than people who don't. The idea is to try, at a very cheap and efficient level, to identify those people who are at high risk.

And another one, what about people who move into the area and move out of the area? Another question is, what about the people who have been in there for 20 years and then retire and get their cancer afterwards?

And the answer to all of these is that we are looking for high risk groups. If we don't find one we haven't lost anything, but if we do, we investigate it.

DR. MASON: If I might exercise the prerogative of the chair, Tom Burke, would you mind taking a few moments -- Tom Burke from the Department of Environmental Protection, State of New Jersey. He and I and Ken Cantor have worked collaboratively for a few years. Tom will share with us some of the other side of the coin. We have been talking about human health issues and industrial hygiene. Here we have a man whose office basically is the extension of the National Environmental Protection Agency with regard to environmental measures. And we have been putting together a data base. Could you share some of your attitudes, frustrations, whatever, with regard to the current project and with regard to the extension? Because you and I have talked about the potential for taking a base such as this, putting it into some sort of a central depository, and if you could talk about that for a few moments?

DR. BURKE: What I will talk about in my formal presentation later is a project that I have been working on with Drs. Mason and Cantor. Essentially, New Jersey, being "cancer alley" and having the highest density of chemical industry in the country, and the highest overall cancer mortality rates, has launched an extensive program to find out about its environment.

We are conducting extensive monitoring of our air, water and biota. We are surveying industry to find out where toxic and carcinogenic substances are being used, emitted, and disposed. We are identifying hazardous waste sites and using the Ames test to get a better idea of what environmental pollutants may cause potential health hazards.

Essentially, what we have been able to do with funding from NCI is construct a data base that will interrelate this information and enable us to develop a statewide profile of community exposure to environmental pollutants. We are hopeful that it will provide a basis for future epidemiological investigations of the role of environment in disease.

My biggest frustration is not with federal cooperation. In fact, the project has attracted a lot of federal interest. The EPA TEAM study which is the largest body burden study ever undertaken chose New Jersey because of our background information on community exposure to toxins. My biggest frustration is probably getting the environmentalists and the epidemiologists to agree on methodologies.

Environmental epidemiology does not have the clearcut nature of the case control occupational study. Clearly, we have identified horrendous potential community health hazards, such as toxic waste dumps and severely contaminated community drinking water supplies. Yet it seems that a good deal of the more medically oriented scientific community is still plodding along with a traditional epidemiological approach, which is ill-equipped to assess environmental hazards.

Traditional epidemiologic investigations take many years, yet we are forced every day to make on-the-spot public health decisions about, say, evacuating people or evaluating environmental hazards. Epidemiologic studies to support these judgments are not available. I think we have to work on quicker methodologies including innovative computer techniques and new approaches to chromosome studies and Ames testing to complement existing epidemiologic methods and provide a more rapid response.

DR. MASON: If I might try to take what has been said so far and perhaps ask Dr. Carnow to comment along the following. You, Tom, like many persons are frustrated because epidemiologic studies take a long time to do. Dr. Carnow, who is now at the University of Illinois, was sharing with me last evening some very interesting things which are currently going on in industry.

I would ask you, Dr. Carnow, to consider the following: Based on what you have heard so far and your understanding of here are two pots, each of several million dollars, do you think it feasible to extend the approach which you are using with your students to have, if you will, a cadre, a truly multi-disciplinary cadre of persons who could, indeed, be funded by one or the other or both of these collaborative programs to facilitate the collection of information in a timely way in which to provide or address emergent questions? Could you comment on that?

DR. CARNOW: What I discussed was the functioning of trainees in our NIOSH ERC, The Great Lakes Center for Occupational Safety and Health at the University of Illinois. We have trainees in occupational medicine, occupational nursing, safety and industrial hygiene. They all attend the occupational medicine clinic and interact in the data gathering process, questioning individuals that are coming to the clinics. Patients are mostly industrial workers who believe that they have an occupationally related disease, with some cases of individuals from communities where they feel that they may have been exposed to or affected by toxic agents. The medicine and nursing trainees gather data on exposures. Where it is felt that exposure may be significant, industrial hygiene trainees not only take histories, but make contact with industrial plants or where communities may be involved, communities, and offer to carry out environmental surveys.

It would seem to me that the utilization of all of these trainees in data gathering, including biological and environmental measurements, could increase considerably the amount of information obtained. This permits us to examine industries for carcinogenic potential of materials or combinations of materials used, and provides environmental measurement data in and out of the plant for use by others.

For at least ten years, some of us have been suggesting that polycyclic aromatic hydrocarbons, trace metals with carcinogenic potential and other such substances be measured at least in those communities where industrial operations are present which produce them. If that is done and we know something about particle size as well as the production and emission data, we can begin to get a handle on the availability of these toxic agents to the communities and develop a baseline which will make possible more elaborate studies. Even without environmental measurements in the air, measurements in the soil or even estimates of levels based on production or emission data could be useful.

While our training is directed toward the development of skills in carrying out environmental and biological measurements in the plants, the measurements in the community are identical, so that this group can be equally useful in carrying out such activities in communities where carcinogenic agents may be present in air, soil and water. Their involvement in such activities, given that there are now 12 centers around the country, could provide substantial input into the collaborative efforts of the NCI, EPA and NIOSH.

I am very encouraged by the quality of the presentations today and certainly feel that collaborative efforts between the three agencies are not only useful, but essential. Our experiences in El Paso and other places where examination of communities revealed significant health impacts from plant emissions suggests that studies carried out in plant which find pathology should almost automatically be extrapolated into the community to make a determination as to whether or not the community, possibly subjected to lower concentrations, but over a longer period of time and containing many individuals at significantly higher risk than workers at the plant, has also been affected.

DR. MASON: Dr. Austin?

DR. AUSTIN: I wanted to comment on the question that you asked about having a multi-disciplinary team trained to investigate these things. The first time I spoke I mentioned our frustration about getting a system that would highlight high risk groups. Well, I think every state has the same frustration about being able to investigate high risk groups when you find them.

I want to draw a parallel between cancer and infectious disease. If you find a plant, a factory, that has an outbreak of hepatitis you can have people there within 48 hours from the Federal Government who are trained, not only about hepatitis but who also have specialized knowledge about water and food, to give you a hand if you need it. But if it is cancer, you are advised to submit a research proposal and maybe in two or three years you can hire and train people to investigate the problem.

I think that it is really critically important when we have high risk groups identified to be able to call on a well-trained multi-disciplinary group, industrial hygienists, epidemiologists, physicians, biochemists, the kind of team that is needed, to come investigate the problem. No state that I know of has been able to put together a well-trained team that sits on their hands waiting for these problems to crop up. And they do crop up almost every month.

DR. MASON: That is the reason that I posed the question that I did. I mean, I will admit to a bias. I firmly believe that these collaborative programs could be better utilized if they would, indeed, have, as we discussed yesterday in another meeting, something like a master agreement which would give us a way in which to put into the field such a team because we are going to be faced with many Love Canals, many other dump sites, many other unique situations where large numbers of persons are exposed to something where we have reason to believe that there might well, indeed, be a long-term effect but we want to get some reasonable baseline data.

DR. AUSTIN: Let me make one more comment. I wanted to point out the one exception to my general statement. That was with Kaposi's sarcoma, in which case there was a federal response but it came from the venereal disease investigation team at CDC.

DR. MASON: Yes, right. Richard, at your institute you tend to be interested in some additional clinical measures on persons, as well as the traditional -- where did you work and how long did you live there and how long have you smoked? Could you envision, do you envision, the potential of coming in with us -- by that I mean any one of the three, one or all three -- and taking an approach similar to what we have just discussed? Can you share with us some of the things that you are currently doing and perhaps from that some discussion as to ways in which to continue to utilize these programs?

DR. EVERSON: Well, I am not very familiar with the background of these programs, and also our intramural program at least in environmental epidemiology at NIEHS is relatively new.

I think one point I might add from the discussion that I have heard so far is that focusing solely on cancer as an outcome for the kinds of exposures you are talking about is probably somewhat narrow. There are going to be effects on fertility and other reproductive outcomes in these same populations, as well as other disease outcomes.

So in a sense you may need a broader base of possible outcomes on which to focus. One of the things that we are attempting to do in our intramural program is to develop new sorts of tests that can be applied to small populations, such as tests of somatic cell mutation that would enable a study of the small high risk groups that you have been describing. But that is a relatively long-range goal of ours and there are not a whole lot of systems on line. Even for chromosomes, there is certainly a lot of discussion about exactly how to go about that.

I think as far as joining in, I think we would have to discuss that over a longer period of time.

DR. MASON: I think that actually what I was interested in is, if we are talking about a multi-disciplinary cadre, and since many of us, you and a number of others, serve at the pleasure of the Secretary, one could, indeed, envision a group like the old EIS officers but now with a broader base that could, indeed, be brought in.

And I think at least from what I have heard so far, no one seems to believe that something like this should not be pursued or that this would not be a reasonable funding mechanism to at least consider along these lines.

Are there other topics that are of specific interest to anyone here? Specific research questions that you believe should be addressed that are currently not funded or perhaps are not funded at a sufficient level in your estimation?

There are some interesting research questions with regard to some of the points you have made, Dr. Everson, with regard to outcomes. Many are interested in the rates of spontaneous abortion among working women as perhaps a sentinel to occupational exposures. There are some very real methodologic issues which I believe are currently being addressed, and maybe one of you from Cincinnati could share that with us.

The last I talked to Bill Halperin there was a plan to use Susan Harlap's data from California to get a better estimate with regard to underlying spontaneous abortion rates. Jay, do you know something on those lines? I think there is some interesting methodologic development. We are making progress on some fronts. Some of the areas are not being touched right at the moment because they are very complex. Could you please tell us what you are doing?

DR. BEAUMONT: At NIOSH we have had a couple of situations where women have been concerned about their rate of spontaneous abortions. The big problem has been standard rates, what do we compare to?

It is difficult to develop a control group at the work site because you have to go back somewhat in time, say, at least three years. And women who are not part of the group that is concerned do not want to answer the questionnaires. And unless we are able to pay them some amount of money, we do not get good cooperation.

So the other choice has been to use standard rates from the Kaiser facility out in California, which have been published by Harlap. The trouble with that data is that while Dr. Harlap has given relative risks in her paper for smoking and alcohol, which are important risk factors, the rates are not specific for combinations of those risk factors. So we cannot stratify the data and really do a proper adjustment.

What we would like to do is get the raw data from Kaiser Permanente, if possible, and either develop standard rates, very specific for these different risk factors, or use the individual pregnancies as units of observation and do regression types of analyses.

We are just in the development stage of this now. We would like to develop some computer systems. So far we do our analyses by hand and we just use the data that has been published by Harlap.

DR. MASON: Okay, are you of the opinion, and I know you don't speak for your Institute, but are you of the opinion that a project such as this, which would benefit any person who is interested in doing occupational epidemiology, could or should be pursued through this mode of collaborative funding either by some in-house persons, which you have expertise there and we have expertise in our group, along with some persons on the outside to sit down and to really work on that? Do you see that as a project that should, indeed, be considered?

DR. BEAUMONT: I personally see it as being very important. I think others in our institute agree and there is a lot of work to be done. I think some collaborative approach would be a very good idea.

DR. MASON: Good. Thank you. Are there any other questions? Comments? Concerns? Yes?

MS. HUTCHISON: I am Cherie Hutchison with the Mine Safety and Health Administration. We are not involved in research per se but we are a regulatory agency with enforcement authority. We do follow research for the purpose of trying to develop regulations and enforce those regulations to try to alleviate some of these problems in the occupational environment.

One of the major problems that I have run into with research agencies and research projects is that they stop too soon. It is very fine that they find an excess mortality in a specific industry and that those workers are exposed to 200 or more chemicals. But the researchers do not know at what level the workers are exposed or how often they are exposed. We get little or no concrete information with which we can work. It is extremely frustrating to say that something is a carcinogen or something is a teratogen and not know or not have the information to do anything with the study.

The regulatory environment right now is quite tight. The need for more concrete data, more dose-response information is very important.

One other problem I would like to address is that I have noticed a tendency for research agencies to fund research beyond the point at which it is useful. In my own particular case, not necessarily with health research, but we do have a lot of money being put into studies on contaminants where alot of information is already known, for example, studies of increased cancer for workers exposed to asbestos. I do not think we need any more research on whether asbestos causes increased mortality. What we need to know now is at what level does the risk increase and what is the magnitude of the increased risk?

DR. MASON: Are there any other comments or questions? Concerns? Then I will make an announcement. Dr. Riggan, a colleague of mine at Health Effects Research Laboratory in North Carolina is the first author of what I believe is going to be a very nice reference work that I offer for everyone's consideration. They are publishing a three-volume tome which works very nicely and extends my earlier work, and will nicely run parallel to what we are doing with regard to rates of change of malignancy at the local level.

He has published 2,600-page tables of rates for the major sites of malignancy for individual counties and states from 1950 to '59; 1960 to '69; 1970 to '78; numbers of deaths and rates, and some concerns with regard to proportionate rates of change by decade. He is currently in limbo with regard to the Government Printing Office. I am proposing that if he stays in limbo past October 1st we will tap the collaborative funding to publish these books because they will provide a very nice resource, reference work, for those of us who are interested in what is happening at the local level. So if anybody, you know, feels like they want more than one -- I don't know how many thousands you are going to print, Wilson -- let us know and we will make certain that you have them. I have here, and I think Wilson brought along some, some 50 copies of it, a brief description of what is in the book and a sample page of tables, so you can see the type of material which is presented. It is an excellent contribution and I commend you for it.

I would like to close this session or else we will never get back on schedule. Thank you very much.

PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

Thursday Morning, September 10

DISCUSSION GROUP

GROUP B - EXPERIMENTAL METHODOLOGY/MODELS

Discussion Leader:
Dr. Thomas P. Cameron
National Cancer Institute

DR. CAMERON: This should be very interesting, because I haven't chaired a group like this before on such short notice, and I'll try to follow the dictates of Dr. Kraybill and open it up.

Now, my understanding is that we're here for this half-hour to try to point our two interagency agreements in new directions -- I won't say better directions. Dr. Kraybill and I had a discussion earlier this morning, which I think is very important. One of the areas we would like to cover in the discussion groups is the quality of some of the projects.

I think, in all fairness, we should consider ourselves among friends. If we have any unease or feeling of being uncomfortable with some of the projects, either in their conception or in their progress, I would like that brought up. Again, I think it behooves us all to be very candid.

With that, I will open the session to the floor. Is there anyone who has an issue that he wants to lead off with? Again, the thrust is critique of the ongoing projects, and new thrusts, and we will try to confine ourselves in this area to experimental methodology and animal models.

I did have one question. Does anyone here actually know how much work has been done with styrene in an experimental capacity, in animal work? What brings it to mind is there was a project which we approved tentatively about two years ago. It was a fairly detailed protocol on transplacental exposure following maternal inhalation exposure with styrene, that was NIOSH-originated.

However, after we approved it in our staff meeting, NIOSH withdrew the project. I think they had second thoughts about the protocol, back at Cincinnati. Is there anybody comfortable talking about that project?

Dr. NIEMEIER: I don't know how comfortable I feel talking about it, because this happened more than two years ago. As I recall, there were some additional data that were found in the Bulgarian literature, which refuted the original Russian findings of increased postnatal deaths, about the time that we were about to award the contract. The main objective of the contract was to confirm the original finding of increased postnatal deaths. A secondary objective was to investigate the possibility of transplacental carcinogenesis.

However, since the original objective was answered and since the secondary objective was much more expensive to investigate than we had anticipated, we decided not to award the contract.

DR. CAMERON: Do you have second thoughts about it? I guess the reason I'm posing the question is that styrene study we just heard about. It's very elaborate. It's a big study, it's been going on for a long time, and I understand they did have problems keeping cohorts together.

But I wondered, with so much emphasis going into that study, was it based on experimental work -- the concern with styrene as a compound, a hazardous compound? And I never did get a feel, myself, that that had been proven.

Dr. Cooper, did you know something about that?

DR. COOPER: If I remember correctly, there was an IARC monograph on styrene, which should bring a good survey of the available data to workers (effective the date that it was produced, which I believe was about two years ago).

DR. NIEMEIER: I guess there are a couple different concerns with styrene, I think, that ought to be mentioned. One is that styrene has a long retention in the body. I think it's unusual, compared with other solvents. Many solvents have that, because they dissolve so well in the fat tissue. But the unusual retention of styrene in particular, I think is important in considering possible carcinogenic effects and the greater body burden, as a result. So I think that's something that ought to be kept in mind, at least.

And there was extensive literature published on styrene, and particularly, there was a monograph that came out -- I guess it was about a year and a half ago on styrene in the Scandinavian literature, the Scandinavian Journal of Work, Health and the Environment, which discussed a lot of this.

So there is a lot of data, I think -- a lot of things that have been going on.

DR. CAMERON: Thank you. Dr. Al Hellman?

DR. HELLMAN: Al Hellman, NCI. What is known on the metabolic breakdown of styrene, as far as in vivo is concerned, and how does the body handle it? In other words, it would seem to me that if that's not known, that is certainly one of the things I would think is important to determine.

DR. BAUMEL: Dr. Baumel, EPA. As I recall the styrene literature, through work on the NIOSH styrene criteria document, the results on carcinogenicity -- and I don't have the details, the results on carcinogenicity were essentially equivocal, and it was difficult to determine as to whether there were any definitive outcomes. There appears to be a gap in the styrene literature relative to styrene oxide, as regards its information, in industrial environments and its formation in vivo from styrene.

There are several groups around the country that could pick up on this and do some very good metabolic pathway tracking on styrene oxide. I think one difficulty is that its formation is relatively rapid, and its stability is somewhat low, so it is difficult to trap styrene oxide and subsequently identify it.

But I'm sure there are techniques that can serve to effectively delineate the important pathway, and I suspect that styrene oxide would play an active role in any carcinogenic effect of styrene. If styrene oxide is definitively identified in a realistic exposure environment, then the concerns may be somewhat heightened.

Results to date with styrene make it difficult to conclude as to whether styrene is in fact carcinogenic.

DR. CAMERON: So Irv, you are saying that there has been work done with styrene, but the results are equivocal in the animal model?

DR. BAUMEL: As far as I can remember, unless there has been new information generated in the last year or so. However, I'm not aware of any.

DR. CAMERON: Let me put you on the spot. Do you feel it would be worthwhile to pursue this.

DR. BAUMEL: I think it would be worthwhile to pursue all possible aspects of styrene oxide research that would help to clarify its role in styrene carcinogenicity.

DR. CAMERON: Oxide specifically?

DR. BAUMEL: The oxide primarily, yes. I'm not sure whether it would be productive to pursue any additional styrene work relative to long-term bioassay testing. I don't know if there will be any additional data that will provide new insights, judging by what's there now.

One might want to look more closely at the experimental design protocols and see if there were any significant weaknesses that could be strengthened and the work repeated. I know there is work going on in the industrial complex on styrene epidemiology that was initiated about a year or two ago. There may be other animal work going on. I'm not aware of it, because I've been away from it, but I would suggest that that be looked into, as well.

DR. NIEMEIER: Rick Niemeier from NIOSH. One that concerns me is related to some recent information that has come from Tom Slaga's group at Oak Ridge National Laboratory. Their research, recently published in Science (213:1023-1025,1981), found benzoyl peroxide to have skin tumor promoting activity. This represents a new class of promoters that has not been identified previously.

I believe that they are studying additional compounds in this family, for instance, methylethylketone peroxide. I don't know offhand whether he's studying styrene oxide, but I believe styrene oxide might be one of those compounds that we should think of as a possible promoter.

It is known from the older literature that styrene oxide does influence metabolism of some of the carcinogens, such as benzo(a)pyrene. I am not familiar with the information on styrene oxide as far as a long-term bioassay, but I think we should begin thinking about these chemicals as promoters and begin testing this hypothesis.

DR. LEWIS: Trent Lewis of NIOSH. There is quite a bit of work on the metabolism of styrene. Dr. Kenneth Leibman of the University of Florida, under a grant from NIOSH -- which he's had for approximately 15 years although through previous agencies, -- has shown that styrene appears to be biotransformed to styrene oxide metabolically. He was unable, however, to isolate or trap this material. The end product of styrene metabolism is mandelic acid, and this metabolite is used to monitor worker exposures, i.e., urinary mandelic acid.

NCI is funding a contract with NIOSH, in terms of work practices for occupational carcinogens, and styrene is the model compound. I think that was reported on last year, or at least one of the previous conferences.

DR. CAMERON: Thank you, Trent.

DR. COOPER: In terms of future directions, I thought the study that was presented this morning on the paper and plywood industry cohorts was extremely interesting, and I assume that the next step -- if any relative risks which are elevated are found in any of the process steps, would be to go in and do a really complete occupational hygiene survey of all the materials to which people are exposed in that particular operational step.

It wasn't specifically stated, but I think it's an obvious next step, and I hope we are going to go on and follow up on any relative risks that are determined, in terms of specific exposure.

DR. CAMERON: Well, I laid out my concern. I am perturbed, and I think I expressed it, that I'm worried about the large scale, long-term, or expensive epidemiological studies, without some backup from the laboratory. I think that's something we should keep in mind.

DR. MILLNER: I'd like to raise that issue. I'm here, I guess, as a fifth columnist. However, many of us in epidemiology do come out of the laboratory, and have -- and our thrust, at this point in time, and it's primarily in grants, is to link your basic laboratory procedures with clinical findings and epidemiologic studies.

This dichotomy of methodology and epidemiology is crazy. We have to look at the exposures, you have to look at the populations, and unless you link all of them, you are not going to have anything in terms of outcome.

And this is a plea that I would like to make. We're encouraging studies that use basic laboratory findings, biological markers, applied to populations, but in well-designed epidemiologic studies. And I think the real problem is the design and the methodology, not just for your laboratory tests, but for your epidemiologic studies.

And unless you can link all of these, you really don't have a leg to stand on.

DR. COOPER: Which comes first, the horse or the cart, or the chicken or the egg? Or do you want them in parallel?

DR. MILLNER: I think they have to be parallel. First, you look for your exposures, you look for your incidence and prevalence. You look to see what there is in descriptive epidemiology. You need to know what exists before you can do your analytical studies and say there is an association that is statistically significant, and that has to be well designed.

But you also have to have laboratory findings, basic laboratory findings -- markers, laboratory procedures, that identify and are correlated with your outcome and disease.

And as we begin to develop this, I think we will begin to have a handle on intervention and possible prevention. And until then, I don't think we really have that much.

DR. BAUMEL: Dr. Millner, in terms of markers, are you expressing preference for short-term, biological markers, relative to the long-term outcomes of carcinogenicity?

DR. MILLNER: I don't think that at this point we should have a preference. We are primarily involved in investigator-initiated grant proposals, which are different from contracts, and it gives the investigator the ability to use whatever interests and abilities he has to come in and do this kind of research.

So is it short-term or long-term? I think we have to be open to that. And if, in peer review, this can be presented in a good study, this will go either way. Do you have any preference? We're open to suggestions.

DR. BAUMEL: I don't think it's a matter of preference, necessarily, but a matter of practicality. In terms of the long-term carcinogenic effects, for instance, the impracticality of getting a representative cohort with a long-term exposure history and an appropriate number or size for that cohort, to have sufficient power in the study, to obtain successful outcome, -- is becoming less and less possible.

But in terms of short-term indicators, whether they're biochemical markers, cell transformation assays, in vitro or in vivo tests, this might, in essence, permit us a shorter exposure period in which to determine the existence of a hazard.

However, that area right now, which EPA Office of Toxic Substances is heavily involved in looking at, in terms of validating short-term testing, is still developmental, but I think there should be some parallelism between the epidemiology research and the short-term testing, so that they can come together in an effective and productive manner.

DR. NIEMEIER: In regard to the problems with wood dust, I have recently completed a draft review of the literature on the etiological agents that may be responsible for the increase in nasal cancer incidence among hardwood workers.

The information that is available on the pulpwood workers, I think, is an entirely different story. I, personally, am planning on submitting some additional studies for interagency funding, in regard to trying to isolate and identify the possible etiological agents in wood dusts.

Most of this planning has come about because of our close relationships with the epidemiologists at NIOSH. Historically, we have been working very closely with them in trying to decide which are the best ways to approach these complex problems.

They are not only feeding to us original information which we use in the design of studies, but the results of our experimental studies are being used in their planning of field investigations. So I think there is a give and take here. We in experimental cancer research cannot ignore the epidemiological results, from the standpoint of being able to apply those to experimental studies and vice versa.

I think that the study on the pulp workers, for example, is very important for us, as experimental toxicologists, to be able to participate in and to attempt to describe which might be the causative agents, and how best, then, can we control the working environment, so that the increased risk factor is described, to some extent, by reducing exposure to those etiological agents.

DR. HEGYELI: Hegyeli, NCI. I would like, really, to join with my remarks to the previous speaker's suggestion, and I relate this to the study that NIOSH conducted on pattern makers, and, as I guess most of you know, a very large medical surveillance study supported by the Ford and the GM motor companies in these people.

But the relative risk in this particular case, according to NIOSH's study, indicate 2.2.5 (sic) times relative risk for colorectal cancer, and not for the nasal or oral cavity, which calls the attention to the type of study that you just mentioned. But detailed animal studies would be needed to sort out what is really the cause, because these wood particles, that most of these pattern makers are dealing with, whether this is laminated wood or hardwood, are large particles, and those lodge, according to the literature, in the nasal cavity and the oral cavity. So the irritation would be in this area.

But there must be some chemical involved in this which would cause -- if the data are really hard enough. That is still, I guess, in question.

Dr. KRAYBILL: Kraybill, NCI. I'd like just to make one or two points: I was gratified to hear your statement about interfacing the experimental world with the epidemiologic world. I don't think we have exploited that.

I think our epidemiological studies start over here, looking at death certificates and mortalities and things of that sort, and mapping, but we ought to explore more fully what's coming out of the experimental area in bioassay.

The other thing I was thinking about, in reading some reports on nutrition and diet, and I read that excellent treatise by Doll and Peto, is the significance of diet and nutrition in relation to colorectal cancer, breast cancer, endometrial cancer, et cetera, et cetera.

However, we're programmed to deal with environmental and occupational stresses, but one should also consider food and diet. In one report, emphasis is placed on protease inhibitors. That work was done by Troll et al. It is a study involving soybean meal in the diet, and maybe we ought to be looking at populations to see what people have a high intake of protease inhibitors, how that relates to a certain type of cancer such as gastric or colorectal.

But I like to see these epidemiological studies, like you said, based on clinical parameters, and then you can take off from there. I think we should emphasize that as a strong recommendation.

DR. CAMERON: I'd like to make two comments. One, Ken Chu at the NCI, started years ago, and I believe he's still following it, to take a systematic look at the bioassay results. He was trying to take each one as they came out, analyze it, and try to convey early warning data, if you would, down the hall to the epidemiologists.

One comment I would like to make before we go further. I do know that there are some industrial people here, and I would just like to extend our welcome. We're very pleased that they're here. I didn't realize that you're all new. Get into the action. Please join in the deliberations and discussions. As you're obviously coming from a different aspect than we do, give us a little push the way you think we should go.

Trent?

DR. LEWIS: Trent Lewis, NIOSH. Tom, I'm concerned about the characterization of the environmental exposures in the first study -- the zinc smelter in Pennsylvania; I think Burt Carnow brought out very well some of the inadequacies -- the respirable size, the composition of the particulate. Unless the environmental characterization is improved, I'm afraid some cause and effect relationships may be made that cannot be substantiated, and may not be valid.

Accordingly I think the investigation must pursue how much of the particulate matter is benzene-soluble, or organic-soluble, and how much isn't, things of that nature. Without that information, I think the study is woefully weak.

DR. CAMERON: John, wasn't a lot of work done on benzpyrenes and attaching them to particulates? Weren't you involved in that activity?

DR. COOPER: Yes, but I think it's not relevant, really, to this discussion. This was dealing with bulk quantities of benzpyrene, in attempts to determine the state of its absorption as opposed to simple admixture for experimental systems.

DR. CAMERON: Okay. Anybody else?

DR. BELLIN: I'm Judy Bellin from EPA. I think one of the problems that we keep facing is how to relate historical exposure to the epidemiology study performed today, and I wonder -- first of all, I'd like to second your invitation to people from industry: here is one area where cooperation with industry, and the knowledge that industry possesses, is of immense value to us.

The second point relates to processes change. While it is important to monitor the industrial exposures now, to relate to our epidemiologic findings, we need to know what the exposures were 25 years ago. How did the process change? And maybe we even have to go back to the lab to recreate what occupational exposure might have been, and to measure what occupational exposure might have been 25 years ago. For this, we really need industry's assistance.

DR. LONGFELLOW: Dave Longfellow, NCI. I'd just like to echo, in that same context, that I think we should not lose sight of the personal hygiene aspect as a route of occupational exposure. One example was raised in the styrene discussion earlier. As you will recall, we hear about the workers cleaning up their exposed arms by sticking their arms down in a vat of acetone up to their elbows, or up to their armpits. Now that's exposure.

The same sort of situation occurs in the industries involved in the metal cutting and grinding operations, where a variety of nitrosamines are known to be formed in the grinding fluids used for lubrication and cooling. I can still remember the gasps of awe from an audience at an IARC International Nitrosamines Conference a few years ago, when it was revealed that significant parts per million concentrations of potent carcinogens could be produced in the grinding fluids during use. The primary route of exposure to personnel was assumed to be inhalation of the atomized fluids, however, a first-hand observation from a member of the audience revealed that food and beverages are frequently placed on the motor housing to keep them warm and that when a worker is cleaning up he will frequently dip his whole arm into the grinding fluid to rinse off metal filings. By such hygiene patterns, a whole new route of significant exposure is created that would not necessarily be apparent from the grinding process itself.

I think there are some messages there for us, when we get down to selecting routes of exposure for the animal bioassay studies and selecting the types of solvent vehicles to use in long- and short-term studies. Let's look to see where the real human exposure is taking place and consider more seriously the solvents used in hygiene as well as the solvents inherent to the operation itself. These may play a significant role.

DR. KRAYBILL: Kraybill, NCI. I meant to make a remark earlier, concerning the woodworkers. We had a meeting in Lyon, France with IARC. Were you at that meeting?

DR. NIEMEIER: No, I wasn't.

DR. KRAYBILL: I see. Well, I think that that monograph is going to come out soon, probably this fall, is that right?

DR. COOPER: It's out.

DR. KRAYBILL: It's out? In that, as I remember, I think there are over three hundred and some chemicals involved. Now, it depends what step you're talking about. The worker in the forest and in the lumber mill gets sawdust and gets exposure to chemicals there. Then if you treat that wood with pentachlorophenol and other chemicals, that's another exposure.

The one I was thinking about is concerning the lumber man, in the woods or in the mill. There are a lot of naturally occurring compounds to which man is exposed.

DR. NIEMEIER: Lumber products?

DR. KRAYBILL: Yes. And I don't know if we have looked at many of these naturally occurring constituents in the wood. That might be something to suggest to the people in the testing program, or bioassay, to take a look at some of these, because some of them could be carcinogens and mutagens. Has that been done?

DR. NIEMEIER: The bioassay program is currently testing some chemicals which are associated with wood dust exposure, cinnamaldehyde, for example. However, there have been some technical problems concerning instability, I believe, in delivering this material to the animals.

We must remember that naturally occurring constituents of wood include not only endogenous chemicals characteristic of the wood at a particular location but also exogenous biological agents such as fungi and fungal products. I think you have to look at the problem from the standpoint of, one, what is the incidence of cancer in the lumberjacks and other members who are handling those materials where the biological agents are present, and second, perhaps more importantly, what type of cancer are those worker experiencing as compared to wood workers with the nasopharyngeal cancer, the plywood workers, have excesses of lymphomas, leukemias, etc. I think, we must consider a number of different carcinogens. I think we may have to address an entirely different class of agents when comparing hardwoods to softwoods.

So it is a lot more complex. I think your 350 chemicals may be an underestimate.

DR. CAMERON: I would like to say something. The point was made just a bit ago about industrial cooperation. I would just like to point out that most of you here know about the NCI-Formaldehyde Institute Collaborative Epidemiological Study. I would like to point out that that concept was initiated by industry; it was the Formaldehyde Institute that came to the NCI. My understanding is that it's most rewarding, and it's going ahead. A large cohort has been assembled, primarily by industry, and it is truly a very interesting study. It could be a real marker -- not a biological marker, but a real marker, in our studies.

DR. FARLAND: Farland, EPA. I would just like to make a comment with regard to methodology, as is is being discussed in this session -- we can do short-term tests on alkylating agents, and on certain other types of potential carcinogens, which we might be interested in, very easily.

This morning, however, we've heard about epidemiology studies with metals, volatile solvents, and physical agents. It is much more difficult for us to do short-term tests with these types of agents, and I think that one direction of our research into methodology, therefore, should be to develop some short-term tests, to be able to screen for these types of potential carcinogens, as well.

Perhaps what we're talking about is getting away from the single-cell cultures, and going into short-term animal models, where you have a more complex biological system coping with these agents. I don't think that it's a simple transition, obviously, from some of the experimental methodology that we have going for us now, to some of the problems that have come up in these epidemiology studies that we were hearing about this morning.

DR. HEGYELI: I would like to mention that, as I see it, the problem we face in short-term testing of chemicals for carcinogenicity is not so much the choice between *in vivo* or *in vitro* tests, but rather, it is the selection of the end points of evaluation.

We face similar dilemmas in our epidemiological studies. Most of our studies to date are retrospective with death as the end point. What we need is more prospective longitudinal studies with genetic markers, chromosomal damage or other short term end points to assess the problem of carcinogenesis in high-risk populations.

DR. CAMERON: Well, that's true. We at NCI are involved in a large prospective study on PBB, a study in Michigan. And it's been underway for quite a while now. But there is a lot of uneasiness with a large, expensive, prospective study like that. We could end up, 15 years from now, with egg on our face.

I mean, I'm with you. I think we should take a chance, in specific instances, but there is a large element of risk.

DR. BELLIN: Again, the studies are tremendously expensive, and I wonder why we are still limiting ourselves to looking at cancer as an endpoint. Are these studies now being broadened, or could they be broadened -- should they be broadened, to include questions on reproductive history, for instance? Should they include reproductive outcomes of both male and female workers and their spouses. I think it might be worth investigating, in the large population groups whether effects are discernible. Often, they can serve as short-term markers.

DR. CAMERON: Agreed. I can't speak for the EPA agency agreement -- I think Dr. Kraybill should. I can speak for the NIOSH agreement. When we deliberate over the new projects for yea or nay, we from the NCI have a basic concern, or problem, for want of another word. We're targeted, or focused, on cancer, and with the understanding that these funds come from the NCI, we have difficulty in accepting toxicological parameters, other than cancer.

I'm not discouraging them as being part of the problem that we would look at, but I guess we have a nagging concern that any one of the projects should somehow relate to the cancer area.

DR. FORD: Ford from International Flavors and Fragrances. I have to say, Dr. Cameron, that I think it is in NCI's interest to keep in mind all of the relatively short-term markers of malfunction, such as teratogenicity, reproductive potency, and neurobehavioral signs resulting from chemical or physical exposures, which, over the course of 25 to 30 years, may result in cancers of the various target systems, but which, after only five of ten years of exposures in people, result in some malfunction.

We were talking earlier about the wood dust and exposures leading to nasopharyngeal cancers. I wondered what sorts of bioassays were being conducted on the products that could be isolated for the wood dusts. It seems to me that those workers are exposed much more than the logger out in the woods to the fine volatiles, by virtue of the fine particulates that are created in finishing. You have at least the 350 chemicals identified in the extracts. Certainly, however, you could look at the major components to see what roles are being played there toward long-term effects. Of course, we in the flavor and fragrance industry have an interest in the specific components, since we isolate many of those for use in long term exposures as flavor and fragrance materials.

DR. COOPER: John Cooper. To try and clarify what you said about endpoints, from my perspective, being a part of the review group, there has, to my knowledge, never been an attempt to exclude any other toxicologic endpoint, only an intent that cancer, as an endpoint, should be one component of studies which are supported.

DR. CAMERON: I'm sorry, I think we had better quit now.

PROCEEDINGS OF THE
SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

Thursday Morning, September 10

Epidemiological and Statistical Session - continued

Session Chairperson:
Dr. George Burton
National Cancer Institute

DR. BURTON: Please, we are about to begin. The first paper in the last session this morning is An Etiologic Study of Respiratory Cancer in Coastal Texas, which is a case control study of lung and laryngeal cancer in Texas among Caucasians. Dr. Patricia Buffler will not be here this morning and the paper will be presented by Tom Mason, who is the co-project officer at NCI.

PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Etiologic Study of Respiratory Cancer in Coastal Texas

Thomas J. Mason, M.D.

National Cancer Institute
Population Studies Section
Environmental Epidemiology Branch
Landow Building, Room 3C29
Bethesda, MD 20205

DR. MASON: The Associate Dean sends her regards, respects, condolences. She called yesterday afternoon and said she couldn't make it.

What I would like to do very briefly because there are no analyses, there are no slides, there are no overheads, and I would like to get back on schedule because I am interested in what Dr. Austin is going to tell us about California, is to tell you basically that this is a large case control interview study of both incident lung cancer and laryngeal cancer in a six county area in Texas which has been identified by those persons who make maps of cancer as having high rates.

What we are interested in doing here, and I can share with you the major hypotheses that are being addressed and put it in perspective with another large study that we are doing, we are concerned about chemical, petrochemical exposures, as well as high asbestos exposures in shipbuilding. As some of you know, this part of Texas, part of which is called the Golden Triangle, which measures very nicely with some environmental measurements which are ongoing by the Environmental Protection Agency, has some exceptionally high rates; has historically large proportions of their work force who are working in particular industries of interest to us. Texas, like other industrialized large states, has made cancer a reportable disease.

The interesting thing to us and at next year's meeting or 18 months hence, if our Division Director holds us to something like that, we will be able to tell you about this study and a parallel study in New Jersey where I am of the opinion that the chemical exposures and the asbestos exposures could, indeed, be the same. The characteristics of exposure will change, not the least of which, as a function of climatic conditions and humidity and other such things, there are more Spanish Americans in our Texas study. And it is the largest study of lung cancer among women that the institute has gotten into. So, hopefully, we will be able to address a number of interesting things concerning the dynamics of the disease; concerning occupational exposures; concerning cigarette consumption, and the potentially most controversial part, does dietary Vitamin A play a protective role with regard to the development of lung cancer in areas where there is ample opportunity for exposure to known carcinogens to the lungs?

We are doing a very detailed dietary analysis collecting information on some 36 or 37 foodstuffs. And it will be interesting when we next meet to be able to share with you whether or not in the United States a similar protective role of dietary Vitamin A controlling for cigarette smoking has been detected.

So with that, I can simply tell you that with regard to lung cancer some 1,064 questionnaires have been completed to date with regard to laryngeal cancer; some 267 interviews have been completed to date. We anticipate the case ascertainment to continue for the next several months and preliminary analyses probably by the spring of next year.

If there are any questions I would be glad to discuss them with you. There are no results, no preliminary analyses. There are some positive points, and this I would share with all of you, we have opened the doors forever to the health care financing administration. Any person who needs access to a sample of the elderly population in the United States, it is free basically for the asking. You can select random samples of persons over 65 years of age by race, by sex, by place. And I think it is a very unique opportunity. It behooves us to move away from the strictly hospital-based studies. We can do population-based studies. We have shown it in a number of instances.

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"ETIOLOGIC STUDY OF RESPIRATORY CANCER IN COASTAL TEXAS"

(NO1-CP-92015-65)

Annual Report October 1, 1980, through September 30, 1981

ABSTRACT

The University of Texas School of Public Health, in collaboration with the National Cancer Institute, is conducting a study to evaluate the relationship of environmental factors to the incidence of respiratory cancer in Caucasian residents of Brazoria, Chambers, Galveston, Harris, Jefferson, and Orange Counties. (Harris County is not included in the investigation of male lung cancer.) The diagnosis periods are July 1, 1976, through June 30, 1980, for the lung cancer cases (except for female lung cancer cases in Harris County for whom the diagnosis period is one year shorter, beginning July 1, 1977) and for laryngeal cancer (for which only male cases are being examined) from July 1, 1975, through June 30, 1980. The cases to be studied are individuals between the ages of 30 and 79 at the time the cancer diagnosis was first made.

In the investigation of male lung and laryngeal cancer, the exposures of primary interest are those relative to occupational exposures with attention also to residential factors and smoking and alcohol histries. In the examination of female lung cancer, residential and occupational exposures are the focus of the study and smoking and alcohol patterns are being assessed. Other risk factors of interest are subjects' medical history, family history of cancer, Vitamin A deficiency and socioeconomic status.

This is a case comparison interview study. Interviews are being conducted with the lung and laryngeal cancer cases or their next of kin, and compeers.

In addition, incidence rates for lung cancer and male laryngeal cancer are being determined for the study diagnosis periods.

INTRODUCTION

The need for the study is based on the high respiratory cancer rates along the Texas Gulf Coast. As this area is the location of several industries, identified as "high risk" (including the petrochemical industry), and as it has a rapidly growing population, it was decided to examine the work and residential experience, other lifestyle characteristics, and medical history of people diagnosed as having respiratory cancer, and comparison subjects selected from the general population.

PROGRESS TO DATE

A. CASE ASCERTAINMENT

1. Hospital Participation

Efforts to obtain the consent of hospitals continued throughout the year.

On Table 1 is shown the number of participating hospitals for each county, broken down into four size categories.

2. Statewide Cancer Registry Program

The contract with the Statewide Cancer Registry Program (SCRP) of the Texas Department of Health was renewed. This stated that the SCRP Staff would continue to ascertain cases in most of the study area hospitals with a hundred or more beds. However, due to internal administrative problems, SCRP found that it was unable to fulfill the terms of the contract and the study staff has ascertained cases in several of the larger hospitals.

SCRP ascertained codes in nine hospitals and partially abstracted 13 others.

A tape listing cases diagnosed outside the study area was obtained from SCRP.

3. Abstracting by Study Staff

This year most of the data collection has been done by one abstractor, with some assistance from a graduate assistant.

Thus far, the study staff have reviewed the records of 25 hospitals and have done part of the abstracting in 13 others (Table 2).

TABLE 1

HOSPITAL PARTICIPATION

County	100 Beds[1]	Yes[2]	No[3]	100-299 Beds[1]	Yes[2]	No[3]	300-499 Beds[1]	Yes[2]	No[3]	500 + Beds[1]	Yes[2]	No[3]
Brazoria	3	3	0	1	1	0						
Chambers	2	1	1									
Galveston				2	2	0	1	1	0	1	1	0
Harris	9	8	1	23	16	8	11	7	4	6	6	0
Jefferson	1	1	0	4	3	1	1	0	1			
Orange	1	1	0	1	1	0						
TOTALS	16	14	2	31	22	9	13	8	5	7	7	0

NOTES

1 - Number of hospitals of this size

2 - Yes - Agreed to participate in study

3 - No - Did not agree to participate

4 - a. One 80 bed osteopathic hospital agreed to participate but only one case was identified - and this only had a radiologic diagnosis and could not be included in the study. The records for 1975, 1976 and 1977 were in the warehouse so were not reviewed.

b. One predominantly black hospital of 107 beds agreed to participate but when the abstractor arrived at the hospital, she was told that there were no cases.

c. The records of a few cases at a rehabilitation center with fewer than 50 hospital beds were packed in heavy boxes. It was not thought to be worthwhile to unpack the boxes as almost all the cases were also identified at other hospitals.

5 - Center Pavilion Hospital, with 236 beds, closed and efforts to locate the records failed. However, it is thought that most of its cancer patients were referred to the hospital from M.D. Anderson. This hospital is not included in Table 1.

Table 2

CASE ASCERTAINMENT - NUMBER OF HOSPITALS

ABSTRACTED BY SCRP AND STUDY STAFF

ABSTRACTORS / NO. OF BEDS	SCRP	STUDY STAFF	SCRP + STUDY STAFF
100	0	13	0
100-299	2	9	8
300-499	4	2	2
500+	3	1	3
TOTALS	9	25	13

NOTE

Four hospitals have yet to be visited

B. INTERVIEWS

1. Interviewers and Territories

Several interviewers were initially hired to cover the six county study area plus eight additional counties and one parish in Louisiana, which made up the "interview area".

Based on estimates of study subjects, interviewers, territories were assigned as follows:

a. Harris, Montgomery, Waller and Fort Bend Counties - 8 interviewers

b. Galveston County - 3 interviewers

c. Brazoria County - 1 interviewer

d. Chambers County - 1 interviewer

e. Jefferson, Liberty, Hardin and Tyler Counties - 2 interviewers

f. Orange, Jasper and Newton Counties and Calcasieu Parish in Louisiana - 2 interviewers

Each interviewer was given standing permission to conduct interviews within a ten mile radius of his/her territory limits. Assignment of interviews was confined to a 50 mile radius of the interviewer's home whenever possible.

Initially, interviewers were hired to work 20, 30, or 40 hours per week, depending upon the projected number of person hours needed to cover a particular area.

Due to the slow pace of case ascertainment and to the fact that it is difficult to fit this type of work into a regular "work week", it was necessary to make two changes in the employment of interviewers. First, effective February 1, 1981, all interviewers' time was based on a minimum of 20 hours per week with the flexibility of working additional hours when the work load permitted. At the same time the number of interviewers was reduced to nine. At present there are seven interviewers.

2. Assignment of Interviews

Assignment of interviews is made by the Field Coordinator every two weeks and is based on the location of potential respondents and the number of cover sheets the interviewer "carried over" from the previous "batch".

A cover sheet is prepared for each study subject assigned to an interviewer, and contains information to help him/her locate and contact the respondent. The interviewer adds information relating to the outcome of the assignment before sending it to the Field Office. (Appendix 1).

Before a batch is assigned, the Field Coordinator reviews the cover sheets available for assignment. Living cases and Medicare controls have priority.

As interviews are assigned, identified eligible respondents are mailed a letter from the Field Office introducing the study and the interviewer assigned to interview that respondent. (Appendix 2). Study subjects without identified eligible respondents must be traced by the interviewer first. When the Field Office is notified of a suitable respondent, a letter is then mailed from the Field Office for the interviewer: in some cases, the interviewer mails his/her own letter to avoid delay.

3. Medicare Controls

The controls for living cases aged 65 and over have been randomly selected from Medicare records on which race is not identified. The Spanish-surnamed persons are identified by the use of the Buechley computer program. To ascertain the race of the other people assigned to them, the interviewers ask some screening questions. These questions were tested in a pretest.

The "Medicare" study subjects are sent a letter from the Health Care Financing Administration, (Appendix 3), which precedes the usual study letter.

One of the screening questions obviously relates to race. If the study subject replies that he/she is Indian (either American or Asian), black or oriental, the interviewer tells him he/she will be contacted in a few days. The Field Office sends a "thank you" letter to the "ex-study subject" indicating that there will be no need to answer further questions.

In all other respects, the interview procedures for these controls are the same as for the other study subjects.

4. Spanish-Surnamed Study Subjects

Earlier this year, it was decided to include Spanish-surnamed cases and controls in the case comparison study. Therefore, some procedures were developed to meet the contingency that the respondents might not speak English.

One of our interviewers was designated as the Spanish speaking resource and all language problems involved in tracing and in arranging and conducting the interviews are addressed to her.

Language has been less of a problem than anticipated and thus far only one interview has had to be conducted in Spanish.

5. Tracing

A very important element of the interviewers' job is tracing the self-respondent or other eligible respondents.

Originally a three hour limit was imposed on the amount of time to be spent tracing a respondent. After a month in the field, this rule was rescinded when it became clear that this was often not sufficient time to locate and identify respondents for whom the only address available in the Field Office could be as much as five years old. Also due to the delay in case ascertainment, the interviewers had time to undertake this additional tracing.

Various methods of tracing are utilized by each of the interviewers. The more rural settings (such as Brazoria, Chambers and Orange Counties) generally have the most informative and helpful resources: utilities companies, post offices, funeral homes and cemeteries, police departments and nursing homes. Small businesses such as service stations, grocery, drug and liquor stores have also proven helpful especially in these more rural areas.

The Privacy Act seems to be better enforced in the urban areas; hence, it is more difficult to obtain assistance from many of the community agencies. Neighborhoods also seem to be more transient so that finding helpful and knowledgeable neighbors can be difficult.

All interviewers rely on city directories, area telephone books, and newspaper obituaries to assist in the tracing process.

6. Reassignment Procedures

Interviews have to be reassigned to a second or third interviewer for the following reasons:

a. A refusal is received from the respondent.

b. A respondent who lives outside the interviewer's territory is identified..

c. When the Field Office has some additional information from a search of the death certificate or another source, or ideas which might help to trace the respondent.

d. Personal circumstances of the interviewer.

"Refusals" are reassigned to a second interviewer except when the Field Coordinator considers reassignment to be inappropriate, as is the case with vehement hostility, or physical or mental weakness. A "persuasion" letter is sent to the respondent (Appendix 4), which can be adapted to address the specific reason for refusal. No attempt is made to convert "second" refusals. However, another eligible respondent is sought for a next of kin interview either after a first or second refusal has been received.

7. Recalcitrant Respondents

During the course of the study, it became apparent that a "prodding" letter was needed to motivate a respondent who was relunctant to schedule an appointment although not refusing to participate. On encountering such procrastination, an interviewer requests that a "prodding" letter be sent from the Field Office (Appendix 5).

8. Contact with Field Office

Efforts have been made to ensure that interviewers have frequent contact with the Field Office and that they do not feel isolated. The following methods of communication are used.

a. "Hot Line" There is a special telephone line in the Field Office to facilitate communication with both respondents and interviewers. Staff are available to answer this line from 7 A.M. to 6 P.M., Monday through Friday. The Field Coordinator is in frequent telephone contact with the interviewers. The "hot line" number is included in all study letters to enable a potential respondent to verify the authenticity of the study, the employment of a specific interviewer, or to leave messages or additional information relevant to the study. Initially, a beeper was rented for the Field Coordinator which would facilitate contact with her during evening hours and the weekends. After a trial period of one month, it was determined that its use could not be justified.

b. "Erudite" A newsletter for and about interviewers is printed every two or three months to keep interviewers apprised of the trials and tribulations of other interviewers, current events in the field of epidemiologic research and the status of interviewers who have left the study. Because interviewers usually work alone and have little support and stimulation, the newsletter acts as a morale-builder for the group. Information relating to interview procedures and techniques, and administrative matters is communicated in memoranda.

c. Monitoring of Interviews On a regular basis, the Field Coordinator observes interviews conducted by each interviewer. She also records the data simultaneously. After the interview, the interviewer is given immediate feedback and this is a good opportunity to discuss other concerns and to obtain some reinforcement.

d. Interviewers' Meetings Several times during the course of the data collection period, all the interviewers meet jointly with the Field Office personnel. These meetings are used to maintain morale by sharing experiences and problems encountered in the field and by allowing interviewers an opportunity to meet other study team members with whom they generally have limited contact.

These meetings seem to stimulate an esprit de corps which is important since the interviewers work in virtual isolation from their colleagues.

The quality of interviewing and tracing is also increased by the sharing of experiences.

In addition, the meetings provide a forum for discussing new procedures, making adjustments in interviewing techniques, and reviewing the status of the study.

9. Quality Control

a. Validating Interviews Ten per cent of each interviewer's completed questionnaires are validated by telephone (See Appendix 6, Verification Form 1 - there is a similar form for next of kin respondents. Three per cent are validated by readministering Section D of the questionnaire and a few of the questions given in the "ten per cent" validation.

b. Monitoring Completed Questionnaires A-1 completed questionnaires are reviewed by the Field Coordinator to identify blatant problem areas such as undocumented missing time, insufficient recording of occupational information and other errors not corrected in the interviewer's editing process. Questionnaires are returned to the interviewers when necessary.

c. Observing Interviewers - (discussed above).

10. Coding Experiences for Interviewers

To increase the interviewers' awareness of the importance of obtaining specific information (and thus the need for good probing), and of recording data clearly, each interviewer was given the opportunity to code some of the sections of the questionnaire. It was agreed that this made the interviewer much more conscientious about the thoroughness of recording and editing procedures as well as the overall neatness and legibility of the questionnaire. Interviewers who had difficulty in grasping the concepts necessary to accurately probe and record occupational information were given an additional opportunity to work with the "Dictionary of Occupational Titles" and the "Standard Industrial Classification Manual" in a limited exercise form.

11. Interviewing Experience for the Coders

It is also believed that an opportunity for a coder to observe an interviewer would increase the coder's understanding of the difficulties an interviewer faces in obtaining the data to be coded. When possible coders have observed an interview.

These joint experiences seem to be rewarding for both the interviewer and the coder.

12. Status of Field Activities

Table 3 summarizes interviewing activities as of September 30, 1981. Case ascertainment is almost complete and 1232 lung cancer and 302 laryngeal cancer cases, eligible for the case comparison study, have been identified.

To date, 1492 interviews have been completed. The response and refusal rates for the lung section of the study are 76.8% and 8.6% respectively, and those for the laryngeal section, 70.7% and 12.9%.

TABLE 3

NCI RESPIRATORY CANCER STUDY

Field Activities Through September, 1981

Type of Interview	Dead Cases	Dead Controls	Living Cases	Living Controls	Totals
LUNG					
Assigned	980	644	219	218	2061
Completed	621	329	118	123	1191
Completed (Excludable Diseases)				23	23
Non-Interviews	145	122	38	54	359
Refusals	54	39	15	25	133
Unable to Locate	52	47	9	15	123
Respondent Outside Interview Area	36	33	14	14	97
Interview Procedures Not Followed	3	3	0	0	6
Cases Identified-Not Assigned	8		25		33
Response Rate	81.1%	72.9%	75.6%	69.5%	76.8%
Refusal Rate	7.0%	8.6%	9.6%	14.1%	8.6%
LARYNX					
Assigned	79	71	211	188	549
Completed	41	34	120	106	301
Completed (Excludable Diseases)				5	5
Non-Interviews	16	27	38	44	125
Refusals	3	10	18	24	55
Unable to locate	8	9	15	11	43
Respondent Outside Interview Area	5	8	5	9	27

TABLE 3 Continued

Type of Interview	Dead Cases	Dead Controls	Living Cases	Living Controls	Totals
Cases Identified-Not Assigned	2		10		12
Response Rate	71.9%	55.7%	75.9%	70.7%	70.7%
Refusal Rate	5.2%	16.4%	11.4%	16.0%	12.9%

C. CODING

1. Coding Manual

Coding of the cover sheet and interview data began December 1980. During the first few months of coding, the coding manual was developed as several drafts were necessary in order to address unforeseen problems. Changes and additions to the coding manual are made in a series of "Coding Notes". Problems are documented by the coders and reviewed by the Field and Project Coordinators.

2. Occupational Coding

The most difficult section to code is the occupational history. "The Dictionary of Occupational Titles" (U.S. Department of Labor) - familiarily known as DOT - is used for the coding of occupations. These codes are supplemented by some developed by the study staff.

The classification scheme of the "Standard Industrial Classification Manual, 1972" (SIC), published by the Office of Management and Budget, is used to code industries.

Some "Occupational Coding Guidelines" have been developed. In the development process, two coding pretests were held:

Pretest 1: The Field and Project Coordinators coded the same 30 questionnaires and the discrepancies were analyzed and decisions made on the preferred codes. These same questionnaires were coded by a doctoral student who had had some experience in using DOT. These codes were then compared to the preferred codes of the study staff by another researcher and the results discussed at staff meetings.

Pretest 2. Three coders each coded another 30 questionnaires after a series of training sessions, and the coding was discussed at meetings of the coders and the Field and Project Coordinators.

Changes and additions to the "Occupational Coding Guidelines" are made in a series of "Occupational Coding Notes".

The problems encountered by the coders are reviewed regularly. The Field Coordinator trains the occupational coders and she and the Project Coordinator answer questions and review coding problems.

3. Quality Control

Ten per cent of all coding is recoded by a second coder. Discrepancies are noted and decisions concerning the correct codes are made by the supervisors. The coding supervisors also recode some questionnaires with priority being given to the occupational coding.

4. Status of Coding

The coding of all sections, except that relating to occupation, has been completed for about 1,000 questionnaires. Approximately 400 occupational histories have been coded.

D. DATA MANAGEMENT

1. Data Input

Data are input at a terminal in the Field Office. One staff member's primary responsibility is the input of the interview data, although he is also a coder. The clinical data and data from the cover sheets, which contain information on the assignment, arrangement and conduct of interviews, are also input by other staff members.

To ensure the quality of data input, the following measures have been and are being taken:

a. There are two abstract forms: the one used for the case comparison study is larger than that used for people eligible for the incidence study only. The input program for the former contains many prompts for the enterer.

b. The interview data are entered twice so the enterer can compare the data.

c. Checks on valid ranges are built into all the input programs.

d. Ten per cent of all data are reentered by a second person.

The detailed abstract forms are entered very shortly after they are received in the office. The shorter forms are a lower priority and about 400 of them have been input thus far.

Cover sheet data are entered as soon as they are available and interview data when the coding is complete and the problems have been reviewed.

It is hoped to have at least 200 questionnaires fully entered ready for some preliminary analyses, by the end of October, but many of the other sections from a larger number of questionnaires, have already been entered.

2. Data Management System

During the past year, the data files have been set up and a system of data management developed. Figure I illustrates the functional relationships among the procedures and data files. The system is complex and only the most important aspects are discussed here and shown on the flow chart.

a. Clinical Data

The medical abstractor (ABSTR) completes a short form for subjects thought to be eligible for the incidence study or a long form for subjects thought to be eligible for the case comparison study.

The information on these forms is entered in batches into the computer via the CRT terminal by a data enterer (FORM 2 and FORM 1).

A program developed by Dr. Buechley is applied to the raw data to identify Spanish surnames. The names are then checked against

Figure 1

NCI RESPIRATORY CANCER STUDY

DATA MANAGEMENT SYSTEM

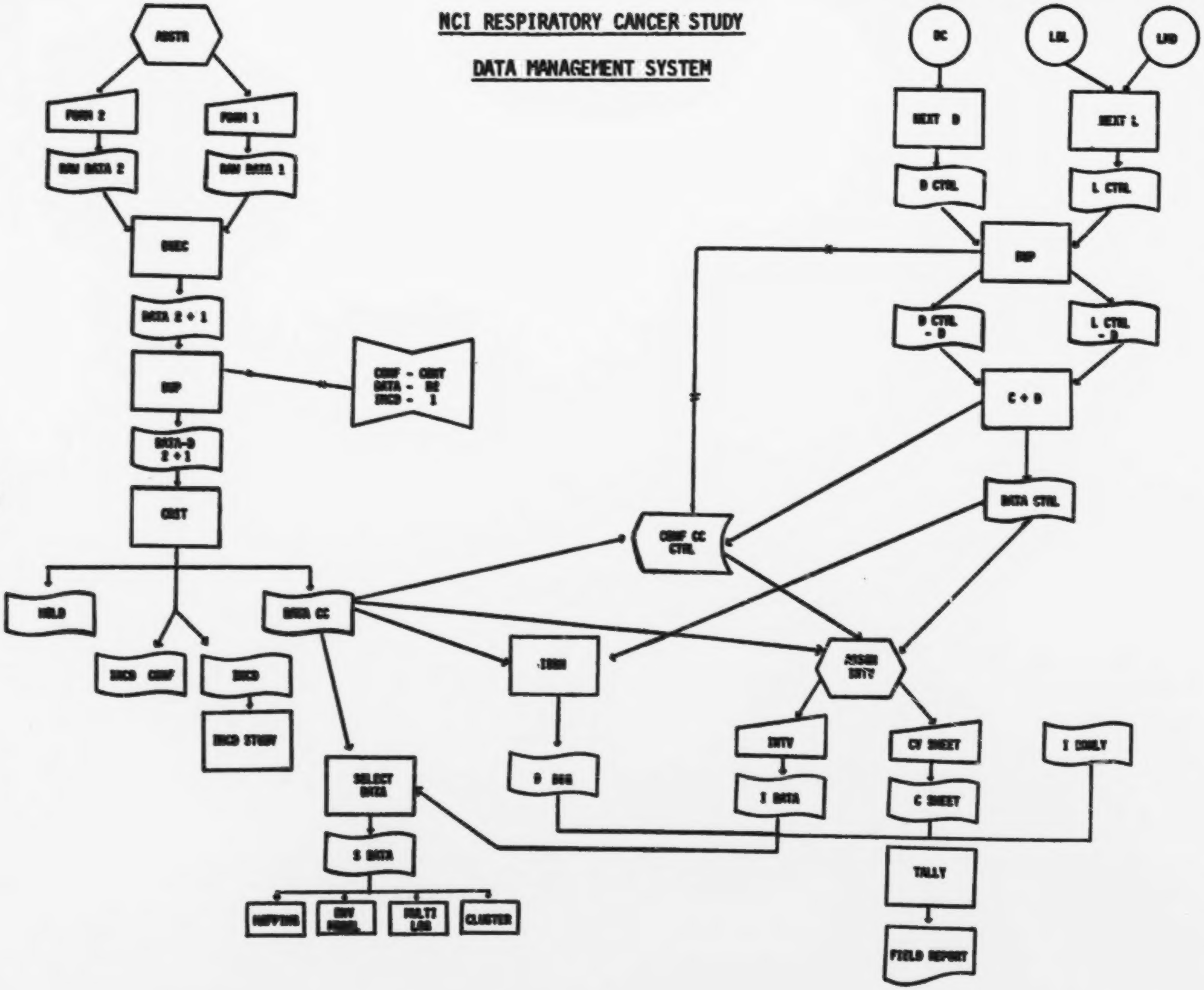
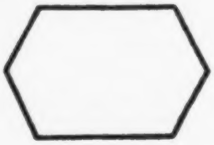
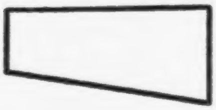
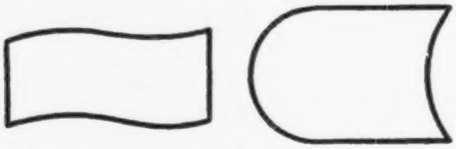
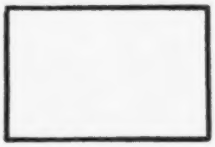
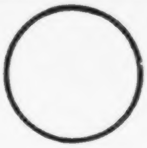
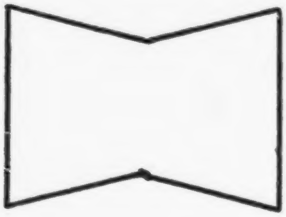
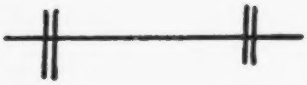

ABSTR
FORM 2
FORM 1
RAW DATA 2
RAW DATA 1
EDEC
DATA 2 + 1
DUP
CONF - CONT
DATA - 82
INCD - 1
DATA-D
2 + 1
CRST
HOLD
INCD CONF
INCD
INCD STUDY
DATA CC
SELECT
DATA
S DATA
MAPPING
ENV
MODEL
MULTI
LOG
CLUSTER
DC
LDL
LHD
NEXT D
NEXT L
D CTRL
L CTRL
DUP
D CTRL
- D
L CTRL
- D
C + D
DATA CTRL
CONF CC
CTRL
IDEN
ASSGN
INTV
P DSG
INTV
I DATA
CV SHEET
C SHEET
I DAILY
TALLY
FIELD REPORT

Figure 1 Continued

KEY

1.		Field Office Source
2.		Form from which data input
3.		Data Files
4.		Computer program
5.		Control tape
6.		Special purpose file-data drawn from other files
7.		Data checked against file

a "special purpose" file to identify any duplicates: this file contains the controls in the confidential file (CONF), the data from Form 2, previously checked for duplicates, and the data from Form 1 which passed the incidence stage of the Criteria Program (CRIT).

i. Criteria Program. This is applied to the data after the duplicates have been removed. Its purpose is to ascertain eligibility for the study. The incidence criteria are tested first followed by those of the case comparison study. The ineligible cases remain in the raw data file (DATA-D 2+1). Those cases which have the potential for becoming eligible (if some unknown or missing data became available) are put in a "holding" file (HOLD). The data of these cases meeting the eligibility requirements for the case comparison study are put into the "DATA CC' file except for the name, address, telephone number, death certificate number and contact information. These data are put in the "confidential" file (CONF-CC, CTRO). The criteria program assigns the complete 9-digit identification number.

ii. Study Subject Number. This embodies the following characteristics of the study subject: whether he is a case, a control or is in the incidence study only, whether he is in the lung or laryngeal section of the study, his vital status, and his age, sex and race. Four digits designate his personal identification number. As part of a strategy to ensure confidentiality, only the "personal" four digit number is stored with the study subject's name.

b. Controls

The control pools come from three sources: the dead controls (DC) are taken from the mortality tapes of the Texas Department of Health, the living controls under the age of 65 from drivers' license records, (LDC) and the living controls, aged 65 and over, from Medicare records (LMD). Batches of controls are selected randomly from these pools using procedures represented by NEXT L and NEXT D for dead and living controls respectively: these include application of the Buechley Program and assignment of the 9-digit SS number. The names are checked for duplicates against the "Confidential" File, and the data are reformatted (C+D). The name, addresses, county of residence and where applicable, the death certificate number of the SS are put into the confidential file and the other data, with the 9-digit SS number, in the DATA CTRL file.

c. Management System

This has been devised to generate information on the status of interviews and facilitates the sampling of controls.

Using information contained in the confidential file and DATA CC and DATA CTRL files, interviews are assigned by the Field Office. Some of the cases identified will not be assigned to an interviewer, either because a physician's consent has not been received, the SSs are too sick to be interviewed and there is no appropriate next of kin respondent, or because they are known to have moved out of the "Interview Area". The SS numbers of these cases are entered into the IDONLY file, together with the non-assigned controls.

Data relating to the assignment and outcome of interviews are entered via the CRT (CV SHT) into the cover sheet data file (C SHEET). The procedure IDBD is used to read the 9-digit SS numbers from the DATA CC and DATA CTRL files and store them in File 9 DIG. This is done to avoid reading the larger data files in the operation of the Management System.

The procedure TALLY is run periodically to generate the following information: the number of interviews assigned and completed within each analysis group (age-sex-race-residence group), the number of non-interviews broken down by reason for non-interviews, and the number of non-assigned cases and controls. The results are tabulated for the monthly Field Report.

d. Interview Data

After the interview data are coded, they are entered via the CRT (INTV) into the interview data file (I DATA).

e. Analysis

When the analysis stage of the study is reached, various selection and preprocessing procedures (SELECT DATA) will be used to read the relevant data from DATA CC and I DATA Files.

These subsets of data (S DATA) may then be accessed by analysis procedures which generate maps (MAPPING), simulate environmental modelling (ENV MODEL), apply a multi logistic function (MULTI LOG), perform cluster analysis (CLUSTER), and perform other analyses of interest to the investigators The confidential file will not be used in the analysis process. For the Incidence Study computations, the data in INCD will be read.

f. Quality Control

Additional procedures not shown on Figure 1 are the data editing procedures. One edit procedure is used to check the Files CONF and DATA, to see if the many dates are in a logical order. Other variables are checked for logical consistency. Another edit procedure checks the 17 different record types in the interview data file (I DATA) for consistency within record as well as between records. These records are also checked for consistency with the coversheet information (C SHEET). The coversheet file too has an edit procedure check for internal consistency. These extensive editing procedures and the input quality procedures discussed above provide timely feedback for data quality control.

When the analysis stage of the study is reached, various selection and preprocessing procedures (SELECT DATA) will be used to select specific records from the clinical data file (DATA) and the interview data file (I DATA).

These subsets of data (S DATA) may then be accessed by analysis procedures which generate maps (MAPPING), simulate environmental modelling (ENV MODEL), apply a multiple logistic function (MULTI-LOG), perform cluster analysis (CLUSTER), and other analyses of interest.

E. ANALYSIS

1. Preliminary Analyses

The work to date on analyses has focused on four areas: preliminary analyses of the clinical data and a subset of the questionnaire data, development of the clustering analyses, description of the census tracts in the region and acquisition and modification of case-control analysis programs.

a. Clinical Data

Analyses of the case comparison medical abstract form were conducted using 1028 completed abstracts; of these 805 were lung cancer cases and 223 were laryngeal cancer cases. Tables 4 through 18 describe these cases in some detail. During the compilation of these tables, data quality was checked and, where needed, data were edited.

b. Description of the Census Tracts

It will be useful in both the clustering analyses and the analyses of smoking to have a detailed description of the census tracts in the study region. This description should be based on the social, demographic and economic characteristics of the individuals in the census tract. The adapted approach is based on the work of W. Parker Frisbie, Dudley L. Poston, Jr. and Isaac W. Eberstein, presented in Frisbie (1976). These authors used 29 variables obtained from the 1970 Census and performed a factor analysis on these variables, yielding seven "social-demographic" factors. These are titled: socioeconomic status, family life cycle, black poverty, migration, Mexican-American fertility, agriculture-female labor force and residential mobility. With the authors' permission these scores have been converted to computer useable form for those census tracts within the six county study region. Using these scores, groupings of census tracts will be made so that the tracts in each group will be homogeneous as to social and demographic characteristics.

c. Analyses of Questionnaires

A subset of the study population has been selected and the questionnaires for these cases and controls are being input to allow for the testing of data editing and analyses. While no attempt to test the study hypotheses will be made using these data, it is important that the programs and procedures for performing these analyses be tried and problems corrected.

Table 4 Vital Status by Type of Cancer

	Lung	Larynx	Total
Alive	240	176	416
Dead	565	47	612
Total	805	223	1028

Table 5 Sex by Type of Cancer

	Lung	Larynx	Total
Male	365	223	588
Female	440	0	440
Total	805	223	1028

Table 6 Race by Type of Cancer

	Lung	Larynx	Total
White	786	209	995
Spanish surnamed	19	14	33
Total	805	223	1028

Table 7 Marital Status by Type of Cancer

	Lung	Larynx	Total
Single	23	11	34
Married	539	175	714
Separated	5	1	6
Divorced	59	12	71
Widowed	159	15	174
Total	785	214	999

Unknown=29

Table 8 County by Type Of Cancer

	Lung	Larynx	Total
Brazoria	139	16	155
Chambers	17	2	19
Galveston	113	11	124
Harris	309	157	466
Jefferson	135	30	183
Orange	74	7	81
Total	805	223	1028

Table 9

Year of Birth by Type of Cancer

	Lung	Larynx	Total
1897-1901	32	3	35
1902-1906	81	26	107
1907-1911	122	36	158
1912-1916	180	51	231
1917-1921	164	51	215
1922-1926	117	27	144
1927-1931	73	15	88
1932-1936	27	8	35
1937-1941	8	5	13
1942-1946	1	1	2
1947-1951	0	0	0
Total	805	223	1028

Table 10 Oncology Code by Type of Cancer

Larynx:

Glottis	125
Supraglottis	38
Laryngeal cartilage	1
Over-lapping	7
Larynx NOS	40
In situ	12
Total	223

Lung:

Trachea	4
Main bronchus	43
Upper lobe	235
Middle lobe	22
Lower lobe	100
Over-lapping	23
Lung NOS	376
In situ	2
Total	805

Table 11 Histology Code by Type of Cancer

	Lung	Larynx	Total
Neoplasm, NOS	1	0	1
Epithelial Neoplasm, NOS	307	16	321
Includes:			
Carinoma	88	7	95
Large Cell carcinoma	35	0	35
Small Cell carcinoma	35	0	35
Oat Cell carcinoma	128	2	130
Papillary and Squamous Cell Neoplasm, NOS	250	204	454
Includes:			
Squamous Cell carcinoma	244	194	438
Adenomas and Adenocarcinoma, NOS	212	2	214
Includes:			
Adenocarcinoma	183	0	183
Cystic, Mucinous and Serous Neoplasm, NOS	4	0	4
Others	28	1	29
Includes:			
Mesothelioma	3	0	3
Total	802	223	1026

unknown=3

Table 12 Grade of Tumor by Type of Cancer

	Lung	Larynx	Total
Well Differentiated	27	53	80
Moderately differentiated	72	65	137
Poorly differentiated	244	17	261
Un-differentiated	191	3	194
Total	534	138	672

Unknown=356

Table 13 Stage of Tumor by Type of Cancer

	Lung	Larynx	Total
In situ	2	12	14
Localized	220	154	374
Regional with direct extension	52	16	68
Regional with node involvement	100	17	117
Regional with direct extension and node involvement	12	7	19
Regional, NOS	10	0	10
Distant or diffuse	324	9	333
Not enough information	76	5	81
Total	796	220	1016

Unknown=12

Table 14 Basis of Diagnosis by Type of Cancer

	Lung	Larynx	Total
Autopsy	11	0	11
Histology	668	222	890
Cytology	126	1	127
Total	805	223	1028

Table 15 First Diagnosis Made Elsewhere by Type of Cancer

	Lung	Larynx	Total
Yes	186	90	276
No	601	131	732
Total	787	221	1008

Unknown=20

Table 16 Oncology Code by Vital Status

Lung:

	Alive	Dead	Total
Trachea	1	3	4
Main Bronchus	5	38	43
Upper lobe	98	137	235
Middle lobe	6	16	22
Lower lobe	39	61	100
Overlapping	5	18	23
Lung, NOS	84	292	376
In situ	2	0	2
Total	240	565	805

Larynx:

	Alive	Dead	Total
Glottis	109	16	125
Supraglottis	26	12	38
Laryngeal cartilage	0	1	1
Overlapping	2	5	7
Larynx, NOS	28	12	40
In situ	11	1	12
Total	176	47	223

Table 17 Histology by Vital Status*

Lung:

	Alive	Dead	Total
Epithelial Neoplasm, NOS	62	245	307
Papillary and Squamous Cell Neoplasm, NOS	91	159	250
Adenomas and Adenocarcinoma, NOS	78	134	212
Other and unknown	9	27	36
Total	240	565	805

Larynx:

	Alive	Dead	Total
Epithelial Neoplasm, NOS	11	5	16
Papillary and Squamous Cell Neoplasm, NOS	162	42	204
Adenomas and Adenocarcinoma, NOS	2	0	2
Other and unknown	1	0	1
Total	176	47	223

*Limited to the major classifications of cells

Table 18 Oncology Code by Histological Type*

Lung:

	Epithelial	Papillary & Squamous	Adenomas & Adenocarcinoma	Total
Trachea	0	4	0	4
Main bronchus	18	20	4	42
Upper lobe	76	84	70	230
Middle lobe	10	5	5	20
Lower lobe	30	31	32	93
Overlapping	4	11	8	23
Lung NOS	169	93	93	355
Total	307	248	212	767

unknown=3
other cell types=35

Larynx:

	Epithelial	Papillary & Squamous	Adenomas & Adenocarcinoma	Total
Glottis	6	119	0	125
Supraglottis	2	35	1	38
Laryngeal cartilage	0	1	0	1
Overlapping	0	7	0	7
Larynx, NOS	3	35	1	39
Total	11	197	2	210

unknown=0
other cell types=13

* Limited to the major classifications of cell type

2. Computer Programs

The following is a list of those computer programs designed for case control or the multiple logistic model which will be used in our analyses:

a. BMDP Stepwise Logistic Regression (PLR)

b. SAS Logistic Procedure

c. Grimson's Case Control Program (CASCTL)

d. Breslow and Day's Case Control Analysis Programs: LOGODDS and STRAT

e. Pickle's Case-Control Analyses Programs: ODDSLOG, FLOGREG, FLOGFIT

The description of the BMDP and SAS programs are provided elsewhere and need not be repeated here. (BMDP-79 Manual and the SAS Supplemental Library User's Guide, 1980 Edition [SAS,1980] for these descriptions).

The Grimson Case Control Program has recently been described in the American Statistician (1981). As used here, it generates odds ratios and test statistics for several strata and combines these values to form adjusted odds ratios and test statistics, including the Mantel-Haenszel and Variance Weighted Odds Ratio and Chi-Square. It is expected this program will be useful in preliminary analyses.

The Breslow and Day Case Control programs, LOGODDS and STRAT, perform analyses on sets of 2X2 tables. Both programs are based on the logistic regression model; LOGODDS is appropriate when there are small numbers of strata, STRAT is used when the number of strata is large. The use of these programs will occur in the final phase of the case-control analyses.

The programs supplied by Dr. Linda Pickle are written as procedures for use with the SAS package. ODDSLOG performs basic data description and provides input to the regression procedures. FLOGREG estimates the maximum likelihood coefficients for the logistic model parameters and FLOGFIT uses the estimates obtained in FLOGREG to evaluate the fit of the model. These programs provide a fairly complete set of tools and will be used in the final stage of the case-control analyses.

3. Clustering

The following procedures will be used as a general approach to spatial-temporal clustering and also to occupational temporal clustering or purely occupational clustering. In determining whether a spatial-temporal configuration exhibits an excess of cases, an "excess" is defined on the basis of expected values computed from an appropriately chosen comparison group.

i. Notation

n = total number of cases

m = total number of controls

R = number of pre-defined mutually exclusive geographic regions of interest

T = number of time-intervals of interest

r,s = indices for geographic regions

t,u = indices for time-intervals

i,j = indices for subjects (cases and/or "controls")

Let w_{tu} be a weighting for the time separation between t and u. Let v_{rs} be a weighting for the spatial separation between r and s.

For example, we may set $w_{tu} = \begin{cases} 1 & \text{if } t=u \\ 0 & \sim \end{cases}$ and $v_{rs} = \begin{cases} 1 & \text{if } r=s. \\ 0 & \sim \end{cases}$ We then assume that w_{tu} and v_{rs} are symmetric in t, u and in r,s, i.e., $w_{tu} = w_{ut}$, $v_{rs} = v_{sr}$.

Further, let r_{it} = the geographic region to which subject i is assigned in time interval t and likewise for r_{ju}. For clarity, we shall write $v(r_{it}, r_{ju})$ instead of $v_{r_{it}r_{ju}}$. For a pair of subjects i,j we define a spatial-temporal nearness score to be $x_{ij} = \sum_{t,u} w_{tu}\, v(r_{it} r_{ju})$, where the summation is over all pairs t,u: $1 \leq t \leq T$, $1 \leq u \leq T$. For example, if $w_{tu} = 0$ or 1, and $V_{rs} = 0_{or}$, as above then

x_{ij} = the number of time intervals in which i and j lived in the same region at the same time.

A total spatial-temporal nearness score for the cases can then be formed by summing over all pairs i,j for which i<j.

$$x = \sum_{i<j} x_{ij}$$

The statistic x is compared to its null sampling distribution, which can be generated by considering random selections of n subjects from among the total of n + m subjects (cases and controls), or by some similar manner, depending upon the sampling scheme.

ii. Other weighting functions various choices of w_{tu} are possible when taking into account the temporal proximity of t and u. For example:

$$w_{tu} = \begin{cases} 1 & \text{if } t=u \\ 0 & \end{cases}$$

OR

$$w_{tu} = \frac{1}{(t-u) + a}$$ where a is a positive constant.

OR

$$w_{tu} = 1$$ for "purely spatial clustering" in the sense that x_{ij} does not take into account the timing of various residences, but it does take into account the length of time at a residence.

Similarly, alternative choices for v_{rs} can be defined. For example:

$$v_{rs} = \begin{cases} 1 & \text{if } r=s \\ 0 & \end{cases}$$

OR

$$v_{rs} = \frac{1}{(r-s) + b}$$ where b is a positive constant.

iii. Comparison of Case Clustering with "Expected" Distribution. Clustering statistics for cases and for "controls" can be computed:

$$x_D = \sum_{1 \leq i < j \leq n} x_{ij}$$ summing over cases ("D" for disease)

$$x_D = \sum_{n+1 \leq i < j \leq n+m} x_{ij}$$ summing over "controls"

Also,

$$x_T = \sum_{1 \leq i < j \leq n+m} x_{ij}$$ summing over cases and controls ("T" for total)

Within each matching strata k, suppose there are n_k cases and m_k "controls." x can be tested for significance by comparing it with its "null" distribution obtained by all possible choices of n subjects, taking n_k from each strata pool of $n_k + m_k$ subjects. There are

$$\prod_k \binom{n_k + m_k}{n_k}$$

such choices. For each choice an X_D is computed and the relative number of times that a computed X_D (from the pooled group) is $\geq$ the observed value for X_D (based on cases only) is counted.

Practically, one must make several hundred or thousand choices at random to simulate the distribution (null) of X_D. Rather than computing every possible subset of the subjects, samples of subject cases may be used to compute an estimate of X_D. Similarily, samples of cases and controls may be used to compute the mean and variance of the null distribution of X_D.

iv. Occupational-Temporal Clustering. For occupational clustering, occupation or industry will be treated in the same way as residence. For "purely occupational clustering," $w_{tw} = 1$ will be used for all time intervals. This will give a "score" for each occupational category (X_r) of interest.

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PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Mortality Study of Fur Dyers and Processors

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MORTALITY STUDY OF FUR DYERS AND PROCESSORS

Introduction

Oxidative dyes comprise a major portion of the permanent hair and fur dyes used in the past century. They contain a mono-aromatic diamine base, which when combined with an oxidizing agent, such as hydrogen peroxide, will permanently color alpha keratin proteins in animal fur and human hair (1-3). At the time of their introduction in 1888, oxidative dyes were considered non-toxic. Since then, paraphenylenediamine, a major component of oxidative dyes, was found to be a potent initiator of dermal and respiratory hypersensitivity among hairdressers, photo-developers and fur processors (4-8). Individual case reports have also attributed cases of aplastic anemia to paraphenylenediamine exposure (9-11).

The carcinogenic potential of oxidative dyes was not recognized until almost a century later in 1969 when Ito reported that toluenediamine, another frequent component of permanent hair dyes, produced hepatocellular carcinomas in rats (12). Greater public concern arose in 1975 when Ames found 89% of 169 hair dye ingredients to be mutagenic (13). The chemicals identified as mutagens included nitrophenylenediamines, nitroaminophenyls, aminoanthroquinones, and azobenzenes which are used in semipermanent hair rinses and in permanent oxidative hair dyes. Subsequent mutagenicity and carcinogenicity studies have corroborated Ames' results (11-29). Table I summarizes the results of tests for mutagenicity or carcinogenicity on hair dye chemicals. Additionally, measurments of metabolites in the urine of tested subjects has shown that the aromatic amines of oxidative dyes penetrate mammalian skin (35-42).

Numerous epidemiologic studies indicate that individuals exposed to hair dye chemicals, either at home or in the workplace, may be at higher risk than those not exposed of developing an increased number of chromosomal breaks, leukemia or cancers of the bladder, breast, cervix, larynx and lung, and other diseases (43-56) (Table II). Other studies, however, found no excess in breast, bladder or all cancer in similar study groups, and among members of a dyers and bleachers union (57-61). It should be noted that, in general, these epidemiologic studies lacked exposure data, especially information documenting types of dyes used and the length, extent, and frequency of exposure to the dyes or dye constituents (62).

A retrospective cohort mortality study of pensioned fur dyers and processors was undertaken in an attempt to evaluate the risk of death due to site specific malignancies previously reported in the literature, as being associated with exposure to the aromatic amine constituents of oxidative dyes.

Process Description

The procedure for converting raw animal skins into marketable furs involves five principle steps (Table III). Members of the cohort have worked in at least four of these operations, dressing, dyeing, finishing and garment manufacturing.

Initially, the skin must be "dressed" or cleaned and conditioned for bleaching or dyeing. Dressing involves cleaning, softening, fleshing, tanning and conditioning of the raw skins. The pelts are soaked in a solution of wetting agents, fat emulsifiers and bacteriocidal agents. After soaking, the furs are "fleshed" whereby the extraneous connective tissue is removed from the leather side of the pelt. In the fleshing process, the skins are dipped in talc then drawn across rotating blades and stationary knives. The skins are then tanned using a variety of tanning modalities including acids, chrome, alum, formaldehyde, vegetable or iron. The type of tanning used is dependent upon the fur being processed and the types of subsequent bleaching or dyeing operations to be performed. After tanning, the skins are placed in rotating drums (drumming) along with hardwood sawdust to absorb the excess tanning chemicals. After drumming the skins are placed in rotating cages (caging) to remove the sawdust. "Drumming" and "caging" are also used to extricate unwanted dyeing, mordanting or bleaching agents from the fur.

Once dressing is completed, the skins are "killed" or treated with solutions of salts called mordants. Mordants have several functions: 1) they may further tan the leather; 2) they react with the fur proteins to prepare it for dyeing; and 3) they react with the dye to produce a desired color. Mordants contain one or mixtures of compounds such as iron.

After mordanting, the furs may be bleached, dyed or both. In most fur dyeing operations, oxidation dyes are used since dyes can produce permanent colors at low temperatures which will not ruin the leather. Oxidation dyes consist of amino, hydroxy, and amino hydroxy derivatives of benzene, toluene, napthalene and diphenyl. Common oxidative dyes used for tinting furs include ortho and paraphenylenediamine, paraaminophenol, 2,4 toluenediamine, 2,4 diaminoanisole and the nitro, chloro and sulfuric derivatives of the above chemicals. For a few furs, such as rabbit or lamb, metallic salts, such as lead acetate or potassium permanganate are used for coloring (2).

To color the pelts after mordanting, the dye is combined with hydrogen peroxide and the pelts are immersed in the dye solution. Color is dependent upon a number of factors including the pH of the solution, the interaction between mordant and dye, and the length of time the furs are soaked in the dye solution. Dyes may also be brushed onto the fur with by hand.

Once dyed, the furs are "finished" to enhance their appearance and marketing quality. Most furs are "glazed" by dampening them with natural gums, then brushed and dried so that the hair lies in the desired direction.

Another form of finishing known as Mouton processing is most often used on lamb pelts and sometimes on rabbit pelts. In this process the furs are first chrome-tanned. The hair is then brushed with a solution of formaldehyde and then smoothed with a hot iron. The heat promotes reaction with the formaldehyde and fur to straighten the inate curl of the fur.

The finished furs are then sorted, matched, cut and manufactured into garments or other products.

Methods

The cohort selected for study consists of 1018 pensioned members of the Joint Board of the Fur Leather and Machinists Workers Union (FLM). Since 1931, the FLM has represented the majority of the fur workers in the greater New York City, New Jersey and Pennsylvania areas. All data used for study were abstracted from pension applications filed with the union by each pensioner.

The FLM was initially composed of five distinct locals (80, 85, 88, 64, and 48) designated according to the types of furs handled, processing operations performed and the geographic location of the shops. In 1960, locals 80, 85 and 88 were combined into a single local, 88. Members of local 85 could still be identified, but it was impossible to distinguish between the members of local 80 or 88, so they were combined for purposes of this study into a single local, 88.

Approximately 40% of the members of local 48 (Pennsylvania) dyed sheep (shearling) and rabbit furs (Table IV). About half of the members were Mouton processors. Another 10% were fur dressers.

The Members of the New York City based "Fur Service Workers" local 64 handle only the finished fur. They are primarily seamstresses, merchants, dealers and auctioneers. There is no apparent exposure to dye or tanning chemicals, however, reports have documented episodes of hypersensitivity among fur service workers due to contact with residual dye intermediates on poorly dyed furs (53).

Members of local 85, a New York City fur dressing local tanned, fleshed, pulled, stretched, and cleaned the leather component of the pelts. These workers were not directly involved with the dyeing process, but were exposed to a variety of tanning chemicals (TABLE V).

The New York City local 88 consists predominantly of dyers of mink, raccoon, muskrat, beaver. This local also includes a small percentage of members from the original local 80 who dyed rabbits and sheep, and about 10% who were Mouton processors (Table VI).

For this study, pensioners were classified into one of the four locals according to the local number designated on the union pension application form. In general, the individual worked in the specified local for most of his career in the fur industry.

In 1952, the FLM Union began awarding normal, early, and disability retirement benefits to its members. To be eligible for normal retirement benefits, the members were required to be an active dues-paying member of the union for at least 20 years and be at least 62 years of age at the time of retirement. To qualify for early retirement and receive a reduced benefit, a member was required to have continuously contributed to the union for at least 20 years, regardless of age. These same criteria are used to determine eligibility for permanent disability retirement, with the additional proof of disability. Permanent disability retirees receive the same pension as normal retirees.

All members of the five FLM locals granted normal, early or permanent disability retirement between January 1, 1952 and December 31, 1977 by the union were selected for study.

Latency and duration of employment could not be obtained because detailed work histories were not available at the union and because many of the shops have closed and the records were unavailable. However, due to the pension eligibility criteria requiring that the individual have 20 or more years in the industry, all cohort members were long term workers.

A modified lifetable analysis will be used to obtain, race, sex, and calendar year specific person years of observation based on length of retirement. The cause-specific mortality experience of the cohort was compared to that expected based on the cause-specific mortality rates of the general U.S. population, and adjusted for age, race, sex, and calendar year.

Of the 1018 individuals who fulfilled the study criteria, vital status will be determined for the entire cohort (TABLE VII). Death certificates were obtained for 520 of the 540 known deaths in the cohort. Since we are currently conducting the mortality analysis, there are no results available for presentation.

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Table I: SUMMARY OF TOXICOLOGIC EVALUATIONS OF MONO-AROMATIC DIAMINE DYES USED IN HAIR AND FUR DYES

	-phenylenediamine						-toluenediamine			diaminoanisole	
	para	ortho	meta	2-nitro-p	4-nitro-o	4-chloro-p	2,4	2,5	2,6	2,4	2,5
Mutagenic Effects:											
increased reversions	x[an]	x[an]	x[abn]	x[bcn]	x[abgn]		x[ag]	x[n]		x[abgn]	x[n]
increased chromosomal breaks, gaps or exchanges				x[e]	x[e]						
increased dominant lethal mutations					x[g]		x[ag]				
Carcinogenic Effects:											
hepatocellular carcinomas		x[d]		x[h]		x[k]	x[af]		x[j*]		
lymphoid tumors				x[a*]	x[a*]						
thyroid tumors										x[k]	
other				x[12dk*]		x[1]				x[3km]	

*non-significant increase

References: [a]Venitt 1976, [b]Palmer 1977, [c]Ammenhauser 1979, [d]Weisburger 1979,
[e]Kirkland 1976, [f]NCI 1979, [g]Blijleven 1979, [h]NCI 1979, [i]Ito 1969, [j]NCI 1980,
[k]NCI 1978, [m]Dybing 1979, [n]Ames 1975

1 urinary bladder tumors; 2 hepatocellular adenomas; 3 malignant neoplasms of the skin

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TABLE II

SITES OF CANCER ASSOCIATED WITH DYE EXPOSURE (EPIDEMIOLOGIC STUDIES)

BLADDER
BREAST
CERVIX
LARYNX
LIVER
LUNG

TABLE III

UNIT PROCESSES IN
THE FUR INDUSTRY

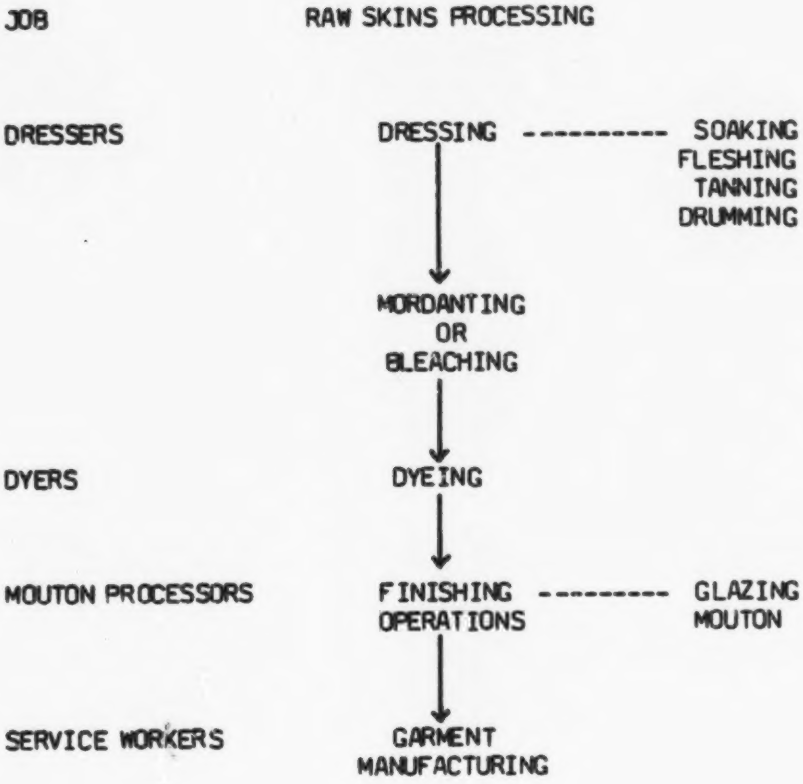
JOB
RAW SKINS PROCESSING
DRESSERS
DRESSING
SOAKING
FLESHING
TANNING
DRUMMING
MORDANTING
OR
BLEACHING
DYERS
DYEING
MOUTON PROCESSORS
FINISHING
OPERATIONS
GLAZING
MOUTON
SERVICE WORKERS
GARMENT
MANUFACTURING

TABLE IV

EXPOSURES AMONG FUR SERVICE AND MOUTON OPERATIONS

Mouton Processors	Finish lamb or rabbit furs by applying formaldehyde and heat.	formaldehyde
Fur Service Workers	Manufacture garments from finished furs; market fur garments.	diamine residue di-imino-quinone intermediate residue

TABLE V

EXPOSURES COMMON TO DRESSING PROCESS

Dressers: Remove excess flesh/fat from the skin with knives; tan leather component of skin

ammonium aluminum sulfate
antimony
copper acetate and sulphate
formaldehyde
sodium and potassium dichromate
silver sulphate
sulphuric, lactic, formic acid

aluminum acetate and sulphate
chromium acetate
ferrous acetate and sulphate
potassium aluminum sulfate
sand
sodium arsenate

TABLE VI

EXPOSURES COMMON TO DYEING OPERATIONS

Fur Dyers: Tint or color furs using semi-automated vat process or by brushing dye onto fur

Diamine Dyes

o- and p-aminophenol
2,4 diaminophenylamine
dimethyl p-phenylenediamine
p-toluenediamine
nitro, chloro and sulfuric
  derivatives of phenylenediamine

Other Dye Chemicals

ammonium vanadate
copper sulphate
bleaching solutions
hydrogen peroxide
lead acetate
potassium permanganate
sodium dichromate
sodium chlorate
wood and vegetable dyes

TABLE VII

VITAL STATUS OF FLM COHORT
1952-1977

Local	Dyers 88 (%)	Dressers 85 (%)	Service Workers 64 (%)	Mouton Processors 48 (%)	TOTAL
Alive	261	29	157	31	478
Deceased	263	141	138	8	540
Deaths without death certificates	(7)	(5)	(6)	(2)	(20) 3.7%
Vital Status Unknown	0	0	0	0	0
Total	514 (50)	170 (17)	295 (29)	39 (4)	1018

DR. BURTON: I have two questions. One is, what is the proportion of men and women among the pensioners? And the other is, in the manufacture of artificial furs, many of which resemble real furs, is there any such exposure?

MS. HARING: We have 78 white females and 878 white males. As far as synthetic furs, I have no idea. I would assume that synthetic furs are not composed of alpha keratin protein, like hair or fur, so regular textile dyes could be used on them, as in cotton manufacturing.

DR. BURTON: Are there any other questions? John Cooper?

DR. COOPER: I have always been a little bit concerned about the hypersensitivity issue which you mentioned. In this sort of investigation, it seems quite possibly that those individuals who are, in fact, hypersensitive to the material, possible absorbing more, possibly being biologically affected to a greater extent, might be at higher risk. Yet, by choosing people who are long-term employees, you may have excluded those people who are most at risk.

MS. HARING: He was referring to the hypersensitivity problem in this group -- the paraphenylene diamine causes hypersensitivity, dermatologic, as well as respiratory sensitivity in some exposed individuals. Now, what he is troubled by is that we are using a survivor population, and we may not be catching those people who drop out early due to hypersensitivity and who also may be more susceptible or greater at risk for certain types of cancer, such as lung cancer.

We also understand that because we are using a pensioner population, we will miss those who died prior to retirement. The Union has some disability records there, but we are not sure exactly how clean the records are. The data we used were very complete.

DR. AUSTIN: Don Austin from California. I am wondering if you have any evidence that pensioners might not be suffering from a healthy worker effect or that might not be involved? My assumption would be that people who are healthy enough to go through an entire lifetime of work might, in fact, still be undergoing the healthy worker effect immediately after quitting work as compared to the general population.

MS. HARING: There have been maybe four of five different studies on pensioners which do not show a healthy worker effect. The all-causes SMR's run closely around 100, maybe 105. Nobody is really quite sure why that happens. As opposed to studies of younger workers where the average all-causes SMR is between 80 to 90. Clearly a healthy worker effect. But studies on retired workers, with few exceptions, find the "healthy worker effect" disappears altogether.

PROCEEDINGS OF THE
SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT COLLABORATIVE AND
OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Epidemiologic Study of Populations Previously Exposed to Hexachlorobenzene

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AN EPIDEMIOLOGIC STUDY OF POPULATIONS PREVIOUSLY EXPOSED TO HEXACHLOROBENZENE

by

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Presented by Henry A. Peters and George T. Bryan at U.S. Environmental Protection Agency Investigators' Workshop in Washington, D.C. in September 1981.

Between 1956 and 1959 approximately 4,000 people in eastern Turkey were exposed to hexachlorobenzene (HCB) when this compound was introduced as a herbicide for the treatment of seed grain that was diverted for food purposes in time of relative famine. There was an estimated 14% mortality during the acute exposure phase, and survivors developed porphyrinuria and red urine associated with intense skin photosensitivity, hyperpigmentation, severe hirsutism and fragility of the skin, peripheral neuropathy, painless arthritis, hepatomegaly, and weakness. Children were referred to as "monkey children" and the syndrome became known as porphyria turcica. Children born to mothers who had ingested the grain passed on HCB in maternal milk and transplacentally, resulting in the death of all children between the ages of two and five. Peters et al[1] reported in 1966 on the chelation treatment of seven unselected cases of hexachlorobenzene-induced porphyria with EDTA. Comparison of clinical courses of patients before and after chelation with EDTA therapy seemed to justify the impression that EDTA was effective in reversing symptoms of HCB porphyria.

We have been able to reexamine 155 patients since 1977 in a field study that included 13 villages in the endemic area. Patient sampling has included some whom we have followed for over 20 years. Clinical symptoms continue to include generalized hyperpigmentation in 84%, marked scarring over areas of previous photosensitive bullae formation in 84%, thickened and tightened skin in 44%, requiring plastic surgery in five patients, significant hypertrichosis including the entire body in 57%, colic in 54%, weakness in 73%, paresthesias in 55%, extrapyramidal symptoms in the form of cog wheeling in 27%, sensory shading in 61%, myotonic reaction in 46%, psychosis in several, and enlarged thyroid adenomas in 39%, which when segregated by sex revealed that 60% of the females had large goiters. Arthritic changes were seen in 70%. Small hands were seen in 69%, and short stature was noted in 47% in those patients who were prepubertal at the time of acute exposure. In addition, sclerodermatous changes were noted.

One hundred eighty-eight total pregnancies were noted in 42 women, followed by 15 fetal deaths and 31 children died in the first several years after delivery, leaving 142 living children. HCB values in milk samples on 40 patients and 45 nonporphyric controls showed an average value of 0.29 ppm and a standard deviation of 0.50. Some decline in HCB values was noted in specimens obtained over a 15-month period. Because of high values of HCB in non-porphyric control specimens in the area of contamination, study of the food chain is in progress.

In our current series of 155 HCB porphyric patients, three had been previously treated in 1961, 1962, and 1963 with EDTA. These three patients showed normal fecal and urine porphyrins and far less hyperpigmentation and scarring than many, but not all, untreated patients. Currently, therapeutic trials with EDTA have been resumed on an exploratory basis in some patients with longstanding HCB porphyria. Rat studies in 1962 revealed that EDTA added to HCB in the food protected the rat from ultraviolet sensitivity. Additional animal feeding experimentation is anticipated.

Patients (155) have been studied clinically, and porphyrins obtained on 121. Seven of these still have active porphyria with a mean uroporphyrin in the urine of 259.5 ug/L, mean of controls 9.35. The uroporphyrin in the urine ranged as high as 1607 ug/L. Correspondingly also in the stool the mean uroporphyrin was 30.7 ug/gm dry weight, in contrast to the control mean of 1.6. The uroporphyrin in the stool ranged as high as 189.2. Of the remaining 114 patients studied, some values were mildly elevated, but the importance of this study could be interpreted in several ways. For example, it has recently come to our attention that similar patients of the same age (approximately 27 years) have been observed in Southeast India with small stature, small hands and arthritis, the "monkey face" with hyperpigmentation, hirsutism; the patients were subsequently suspected of having porphyria, but the porphyrins were negative. We are attempting to obtain specimens from these patients in India to determine if they have an increase of HCB in the tissue and whether or not the Indian government used HCB as a fungicide or imported Surmesan or Chlorable from several European sources. The area in India is at Karigiri and was observed by physicians at the Schieffelin Leprosy Research & Training Centre located in Southern India. In Turkish patients it is also of interest that the porphyrin precursor, delta aminolevulinic acid (ALA), in 26 of 45 (58%) patients so far studied was quite elevated. The porphobilinogen was, however, normal. The mean ALA of the 45 patients studied was 4.5 mg/L, in contrast to the controls which was 3.53. The porphobilinogen was normal; i.e. mean was 0.41 (S.D. 0.17), and for controls was similar: 0.39 mg/L (S.D. 0.14). These porphyrin precursors may be elevated in acute intermittent porphyria and could correlate with the neurologic symptoms that we are observing in the Turkish porphyria group.

During the past year, a 90-day subchronic feeding and toxicokinetic study of HCB in rats, mice, and hamsters was completed. The tissue levels and the rate of disappearance of HCB in fat and liver of both sexes of these species after oral administration was measured. After a 90-day treatment period, HCB fat levels decreased exponentially, and the calculated half time of HCB in fat varied (41-53 days) with the dose and animal species. The growth of test or control animals were similar. Necropsy showed more prominent lesions in males and included marked hepatosplenomegaly, and congestion in the gastrointestinal tract and in all other abdominal and thoracic organs. Thymus glands, spleen, and lymph nodes were usually enlarged. Histologic examinations revealed congestion and microhemorrhages in the central nervous system; degenerative and precirrhotic changes in the liver; hyperplastic lymphocytic proliferations in the lymphatic centers and thymus glands, with hepatic infiltration; and severe hyperemia and degenerative changes in the epithelial lining of renal tubules. A two-year chronic toxicity study of HCB in male and female rats is currently in progress.

Acknowledgements: This research is supported by EPA Contract No. 68-01-5002 and NCI/EPA Interagency Agreement No. Y01-CP-80205; and by NCI Grant CA 14520 (WCCC Core).

Our special thanks go to Barbara Darcey and Margaret Olson, without whose help we could not have written this paper, and to Joy Savides for her clerical supervision.

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SPEAKER: I am curious if you have any theories as to how EDTA works?

DR. PETERS: Any theories as to how EDTA works. Well, we have many theories. We believe that it does complex with zinc and other heavy metals. It is used, as you know, in the treatment of lead poisoning. Our particular theory holds that porphyria is due to an enzymatic block, and, incidentally, can be identified in the acute porphyric. This enzymatic block may be due to an excess of medications for some reason or another.

This is a condition which in the genetic type of porphyria is frequently brought on by medications like barbiturates and sulfur drugs. So we believe that EDTA unblocks the pathway. We have some evidence that is maybe what happens.

It also probably cuts down on photosensitivity by complexing medications even in the skin, because that was a very striking feature in the acute phase.

DR. KRAYBILL: Kraybill, NCI. Have you titrated this project looking for cancer? Are there any skin cancers or cancers at other sites?

DR. PETERS: We have not seen any clear evidence of this. We are looking for evidence. We do have, of course, a 60 percent incidence in the women of thyroid disease that runs out to 33 percent if we average in the men. But whether these will become malignant or not, that is hard to say. Carbal has shown that this does happen in rats and other animals. And we do have some preliminary rat data.

DR. KRAYBILL: It would be quite satisfying to see some effects here in terms of cancer because it is an NCI-sponsored project.

DR. MORRIS: I didn't catch all the conversation, but did you mention that we do have animal data that would suggest HCB certainly in the hamster and the mouse and now in the rat. HCB is a carcinogen. So we have some evidence biologically in other systems.

The other thing I think you might mention too, Henry, is about our work in compounds other than EDTA in terms of perhaps some combined therapy.

DR. PETERS: Yes, we are utilizing the rat model to try a number of compounds, and one of them, of course, is EDTA. There has been some evidence that levels are influenced by cholestyramine resin, something else which has been utilized in cutanea tarda porphyria. These will be explored further.

DR. CARNOW: Carnow, University of Illinois. Since dioxin also may cause porphyria cutanea tarda, would you suggest that possibly people who are so affected might be helped by EDTA? I am really curious on how you zeroed in on EDTA as a possible treatment?

DR. PETERS: Initially, in the acute patients -- I had a patient who was literally almost dead, and because she had severe peripheral neuropathy I gave her dimercaprol propranolol. And that seemed to work. So it was a natural that we would try this too.

DR. CARNOW: Fascinating.

SPEAKER: Did the Turkish Government make any systematic effort to discourage breast feeding?

DR. PETERS: There has been a lot of work in this area, largely by the Haceteppe University. I don't believe there has been any definite attempt in this area. On the other hand, this is mixed in with religion and everything else, as you know. This is the tendency now, to discourage it completely. And I think this data is going to help because it is obvious that the compound itself has not disappeared from human fat and is still showing up in very high amounts, comparatively, in the milk.

We have also been able to demonstrate that in surveying milk samples throughout lactation the levels go progressively down.

DR. BURTON: I think we will have to cut the questions at this point due to the fact that we are running late. Thank you very much, Dr. Peters.

DR. PETERS: Thank you very much.

PROCEEDINGS OF THE
SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Incidence of Cancer as Related to Industrial Emissions in Contra Costa County, California

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THE RELATIONSHIP OF AIR POLLUTION TO LUNG CANCER IN CONTRA COSTA COUNTY

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Resource for Cancer Epidemiology Section
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Contra Costa County, (C.C.Co.), in the northeastern part of the San Francisco Bay Area, is heavily industrialized with five major petroleum refineries and many chemical plants. In 1977, the County Health Department was awarded an EPA grant to study the relationship of industrial air emissions and cancer in that county, in cooperation with the Resource for Cancer Epidemiology (RCE) in the State Health Department. The RCE was to provide cancer incidence data from the local SEER Program area which includes C.C.Co. Shortly thereafter the study became embroiled in a political controversy and the grant was returned by the C.C.Co. Board of Supervisors with a request that the study be turned over to the State. As a result, the RCE took over the conduct of the study, essentially as originally conceived, with four study components: 1) a chemical and biochemical characterization of ambient air particulate matter in the county; 2) a study of cancer incidence, focusing on the industrial and nonindustrial areas and on the correlation with air pollution levels; 3) an effort to monitor cancer incidence in various occupational groups, and 4) a case-control study of any unusual cancer findings revealed in the incidence studies.

The occupational monitoring aspect of the study was expanded through a State appropriation and, although it resulted in significant findings in other areas, contributed no significant results in C.C.Co. and will not be discussed further. The case-control study is in progress and data are not yet available.

METHODS

Particulate air pollutants were collected for a one-year period: November, 1978 through October, 1979. Five permanent sampling stations in or just outside C.C.Co. were supplemented by the addition of ten temporary stations (Fig. 1). The stations were placed in an attempt to characterize the air breathed by the population. Thus, stations were widely spaced in sparsely populated areas and were closer in more densely populated areas. In addition, stations were not placed adjacent to emission sources or major traffic thoroughfares and prevailing windflow patterns were taken into consideration. Hi-vol glass filters were collected every sixth day and particulate matter analyzed and prepared as averages for each of the three meteorologic seasons and for the year. Analyses were conducted for total suspended particulates (TSP), benzene-soluble organics (BSO), sulfates (SO_4), nitrates (NO_3), lead (Pb), selected polycyclic aromatic hydrocarbons (PAH) and mutagenicity using the Ames test. Values from each of the 15 sampling stations were used to compute estimated values for each of the population centroids of each of the 115 census tracts in the county. In addition, a computerized modeling technique was used to map the county by isopleths into four levels for each pollutant (Fig. 2).

The preliminary analysis of cancer incidence for the period 1972-1975 for all sites combined, lung and bronchus, stomach, prostate and lymphoma, showed a major difference in the incidence of lung cancer between the residential and industrial areas. Subsequent analyses were limited to lung cancer. For the next analysis, the age-adjusted and the age-, race-, sex-specific incidence rates of lung cancer were computed for each year and for two five-year intervals for the period 1969-78 for industrial and nonindustrial areas. All census tracts both zoned and used for heavy industry were aggregated to form the industrial area (Fig. 3). Twenty-two percent of the white and fifty-four percent of the black population was included in the industrial area. The remaining portion of the county constituted the nonindustrial area. Age, race and sex components for each census tract were computed using the 1970 U.S. census and a 1975 countywide census. A final analysis further refined the tract-specific population estimates using 1980 census data, and vital statistics from each census tract. It covered the period 1970-79 and relied upon individually reviewed and verified case reports of lung cancer by census tract.

Pearson product moment correlation coefficients were computed for comparisons between estimated annual values for each air pollution constituent for each census tract and the 5- and 10-year average annual age-adjusted incidence rates for cancer of the lung for white males and white females for each census tract. Two census tracts, one a Naval base and the other a retirement development were considered atypical and were excluded from the correlational analyses. In addition, certain census tract specific attributes from the 1975 census such as median family income and percent of household heads living 20+ years in that census tract were correlated with cancer incidence and air pollutants. Partial correlations, controlling for certain census tract attributes were also conducted.

FINDINGS

Preliminary lung cancer incidence rates for the industrial and nonindustrial areas of the county show a widening difference for each race and sex over the period 1969-78. Final incidence rates for the period 1975-79 show a significant excess of lung cancer in the industrial area residents (Table I). The magnitude of the excess is 39% for all races and both sexes combined. The explanation for this excess is being sought through a case-control study but clues to the cause may be provided by the correlation of the rates with air pollution constituents and census tract characteristics.

There was a good correlation (Pearson product moment correlation coefficients: .64-.87) between the individually measured PAH's and BSO. For the Ames mutagenicity test without liver homogenate (-S9) the correlation with BSO was .24 and ranged from .12 to .71 for the PAH's. Corresponding values for the Ames test with liver homogenate (+S9) were similar (BSO = .28; PAH's = .02-.60). Pb correlated well with BSO (.78) and less well with both mutagenicity tests (-S9 = .24; +S9 = .28).

Individually characterized PAH's accounted for about 2% of the total mutagenic activity in the air, which perhaps accounts for the relatively low correlation between mutagenicity and BSO or mutagenicity and the individual PAH's.

Both the high correlation between Pb and BSO (.78) and the similarity of their geographic contour maps suggest that the automobile is the major source of these pollutants. The lower correlation with mutagenicity to Pb, BSO and the PAH's and the different contour configuration for mutagenicity suggests that several different sources contribute to mutagenicity.

The correlation coefficients based on the computed values for 113 of the 115 census tracts show very similar relationships to those based on the 15 monitoring stations (Table II). Thus, while the correlation between Pb and BSO for the 15 monitoring stations was .78, for the 113 census tracts the value was .70. Likewise, the 15 station correlation between BSO and one of the PAH's (benz(a)anthracene) was .87, and for the 113 census tracts the value was .89. For the 15 stations the correlation between BSO and the two mutagenicity tests (with and without S9, respectively) were .28 and .24 while the correlation based on 113 census tracts was .39 and .28, respectively.

The correlation of census tract specific average annual lung cancer rates for 5-year periods (1970-74, 1975-79) were examined for white males and white females. Of six possible correlations from the 4x4 table, only one differed significantly from zero (Table III). Thus, it was concluded that the five-year lung cancer incidence rates by census tract were too unstable to use in the correlation analysis and only ten-year rates were used. The correlation between the 10-year census tract specific lung cancer incidence rates for white males and white females was .33 ($p < 0.08$) for all tracts and .50 ($p < .0001$) with the two atypical tracts removed.

The 10-year census tract specific lung cancer incidence rates were significantly correlated with only one measure of particulate air pollution, that of SO_4 (Table IV). The correlation coefficient for white males was .46 ($p < 0.0001$) and for white females was .16 (NS). Controlling for the percent of households in the census tract in which the head of the household had resided in the unit for 20+ years, as determined in the 1975 Contra Costa County census, reduced the correlation slightly for males. Controlling for the percent of census tract residents of Spanish origin did not affect the correlations.

Controlling for the percent of blue collar workers significantly reduced the correlation between SO_4 and lung cancer for males. This reduction was from independent effects of both the skilled and unskilled laborer components of blue collar workers. Controlling for the percent of households below the poverty level reduced the SO_4-lung cancer correlation only moderately. Controlling for education or income variables reduced or destroyed the correlations for males.

DISCUSSION

Several conclusions may be drawn from the data gathered in this study to date. It is apparent that an excess of lung cancer exists in the industrial portion of the county. This excess has developed over the past decade.

Mutagenic activity, as measured by the Ames test, is identifiable in the particulate matter in ambient air in Contra Costa County and is best associated with the distribution of BSO and Pb, suggesting mostly automobile sources.

The ten-year occurence of lung cancer in white males only is weakly but significantly associated with the distribution of SO_4 particulate matter in the ambient air. This association is reduced equally by controlling for the percent of skilled, or unskilled, laborers in each census tract. Controlling for both factors combined nearly destroys the observed association.

The source of SO_4 in the air is almost exclusively from oil refining, chemical manufacture or oil combustion for electrical power generation. The association of lung cancer in males with this factor might suggest a carcinogenic effect from past petrochemical emissions were it not for three factors: First, the current mutagenic activity in the particulates is distributed differently. Second, the association does not occur in females. Third, the association appears to be mediated through a residential pattern of laborers which correlates well with the distribution of SO_4 (.67; $p < .0001$).

Although these data permit no conclusions regarding the causes of lung cancer in the county, they suggest that a significant contribution to incidence by blue collar workers residing in areas of close proximity to petrochemical plants will be demonstrated in the current case-control study.

TABLE I

FIVE-YEAR AVERAGE ANNUAL AGE-ADJUSTED[1] INCIDENCE RATES FOR CANCER OF THE BRONCHUS AND LUNG, INDUSTRIAL AND NONINDUSTRIAL AREAS OF CONTRA COSTA COUNTY 1975-79

GROUP	INDUSTRIAL AREA	NONINDUSTRIAL AREA
MALES	101.2 (89.4 - 113.0)[2]	76.9 (71.2 - 82.6)[2]
FEMALES	41.7 (34.6 - 48.8)	30.3 (27.2 - 33.4)
WM	108.6 (94.5 - 122.7)	77.9 (71.8 - 84.0)
WF	44.7 (36.5 - 52.9)	31.0 (27.7 - 34.3)
BM	92.9 (65.9 - 120.0)	70.7 (40.7 - 100.7)
BF	37.2 (21.5 - 52.9)	15.6 (3.8 - 27.4)
TOTAL	69.4 (62.7 - 76.1)	50.0 (47.1 - 52.9)

[1] RATES ARE ADJUSTED TO THE 1970 U.S. STANDARD.

[2] NUMBERS IN PARENTHESES ARE ± 1.96 STANDARD ERROR.

TABLE II

COMPARISON OF THE PEARSON CORRELATION COEFFICIENTS FOR THE MEAN ANNUAL MEASURED VALUES FOR 15 MONITORING STATIONS AND FOR COMPUTED VALUES FOR 113 CENSUS TRACTS, CONTRA COSTA COUNTY, NOVEMBER 1978 - OCTOBER 1979

COMPARISON	15 STATIONS	113 CENSUS TRACTS
BSO vs Pb	.78	.70
BSO vs Mut (-S9)	.24	.28
BSO vs Mut (+S9)	.28	.39
BSO vs BAA	.87	.89
BAP	.80	.84
BGP	.64	.71
CHR	.83	.84
BSO vs SO_4	.19	.17
TSP vs NO_3	.64	.75

TABLE III

CORRELATION OF THE CENSUS TRACT SPECIFIC, FIVE-YEAR AVERAGE ANNUAL AGE-ADJUSTED LUNG CANCER INCIDENCE RATES IN CONTRA COSTA COUNTY FOR WHITE MALES AND WHITE FEMALES[1,2]

	WM 1970-74	WM 1975-79	WF 1970-74	WF 1975-79
WM 1970-74	1.00	-	-	-
WM 1975-79	.17	1.00	-	-
WF 1970-74	- .02	.14	1.00	-
WF 1975-79	.23	.54**	.05	1.00

$^{**}p < 0.01$

[1] CORRELATION OF THE 10-YEAR RATES FOR WM VS WF = .50 ($p < 0.0001$)

[2] 113 CENSUS TRACTS

TABLE IV

CORRELATION OF THE TEN-YEAR (1970-79) AVERAGE ANNUAL AGE-ADJUSTED LUNG CANCER INCIDENCE RATE TO PARTICULATE AIR POLLUTION CONSTITUENTS[1], BY INDIVIDUAL CENSUS TRACT[2], CONTRA COSTA COUNTY, CALIFORNIA

AIR POLLUTION CONSTITUENT	VS	AVERAGE ANNUAL INCIDENCE RATE (PER 10^5)	
		WHITE MALES	WHITE FEMALES
Mutagenicity (-S9)		-.01	-.07
Mutagenicity (+S9)		.05	-.01
BSO		.11	-.09
BAA		-.05	-.18
BAP		-.09	-.18
BGP		-.13	-.16
CHR		-.03	-.14
TSP		.20*	-.07
Pb		.11	.06
NO_3		-.09	-.24*
SO_4		.46****	.16

---

[1] Mean Annual Value, November 1978 - October 1979

[2] 113 Tracts

* $p < 0.05$

**** $p < 0.0001$

TABLE V

CORRELATION BETWEEN SULFATES AND THE 1970-79 TEN-YEAR AVERAGE ANNUAL LUNG CANCER INCIDENCE RATES, BY INDIVIDUAL CENSUS TRACT,[3] CONTRA COSTA COUNTY, CONTROLLING FOR SEVERAL CENSUS TRACT CHARACTERISTICS[1]

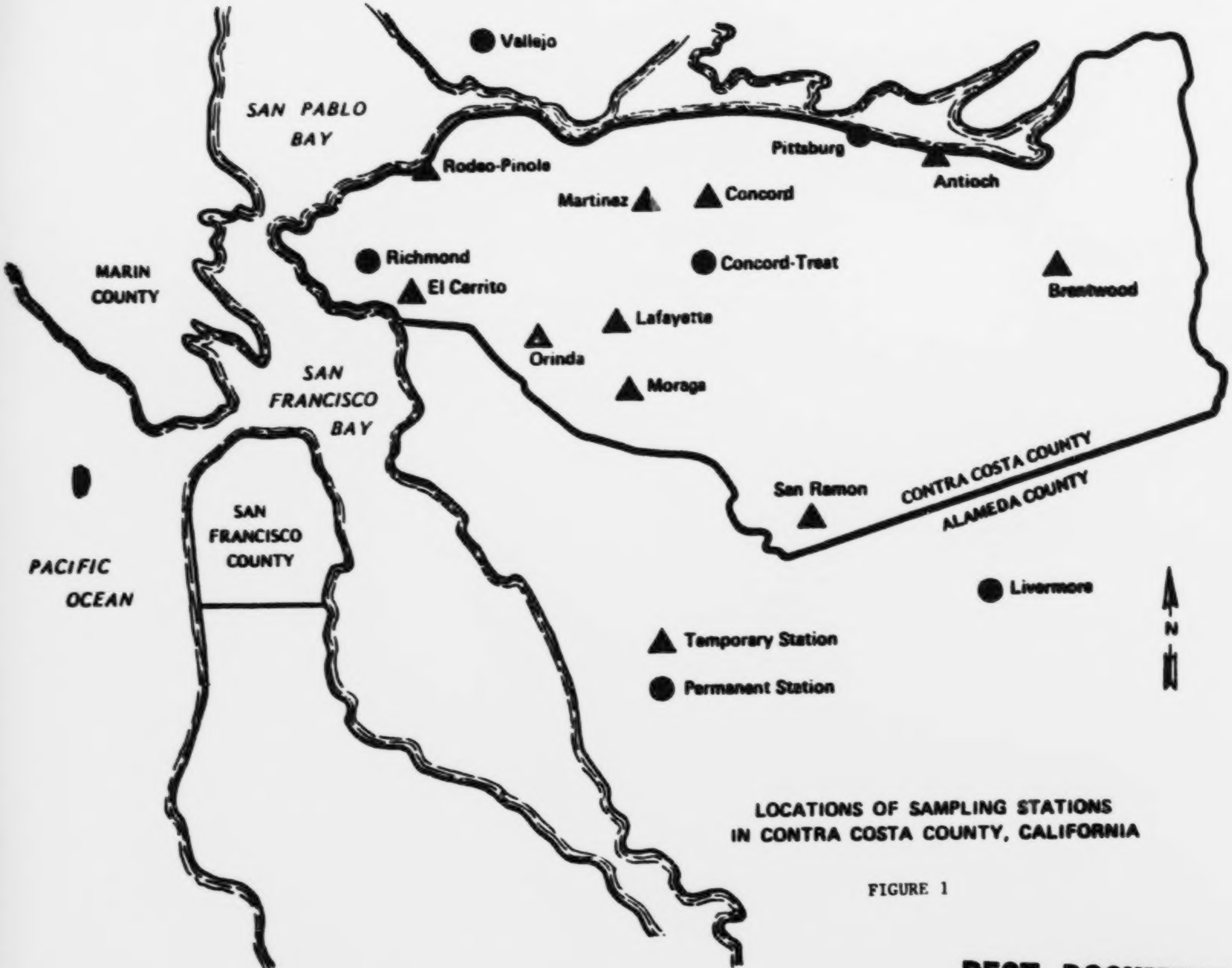
	CONTROLLING FOR:	SO_4 vs WM	SO_4 vs WF
1.	Nothing	.46****	.16
2.	Head of household resident of census tract for 20+ years	.46****	.13
3.	Percent Spanish origin	.42***	.19*
4.	Percent blue-collar workers[2]	.21*	.02
	a) Percent unskilled laborer[2]	.29**	.04
	b) Percent skilled laborer[2]	.29**	.09
5.	Percent of households below poverty level	.30***	.07
6.	Median family income	.21*	-.03
7.	Median school completed by head of household	.17	-.06

[1] Data from 1975 C.C. Co. Census

[2] Data from 1970 U.S. Census

[3] 113 Census Tracts

*	$p < 0.05$
**	$p < 0.01$
***	$p < 0.001$
****	$p < 0.0001$


Vallejo
SAN PABLO BAY
Pittsburg
Rodeo-Pinole
Martinez
Concord
Antioch
MARIN COUNTY
Richmond
Concord-Treat
El Cerrito
Brentwood
Lafayette
Orinda
SAN FRANCISCO BAY
Moraga
San Ramon
CONTRA COSTA COUNTY
ALAMEDA COUNTY
SAN FRANCISCO COUNTY
PACIFIC OCEAN
Livermore
N
▲ Temporary Station
● Permanent Station

LOCATIONS OF SAMPLING STATIONS
IN CONTRA COSTA COUNTY, CALIFORNIA

FIGURE 1

CONTRA COSTA

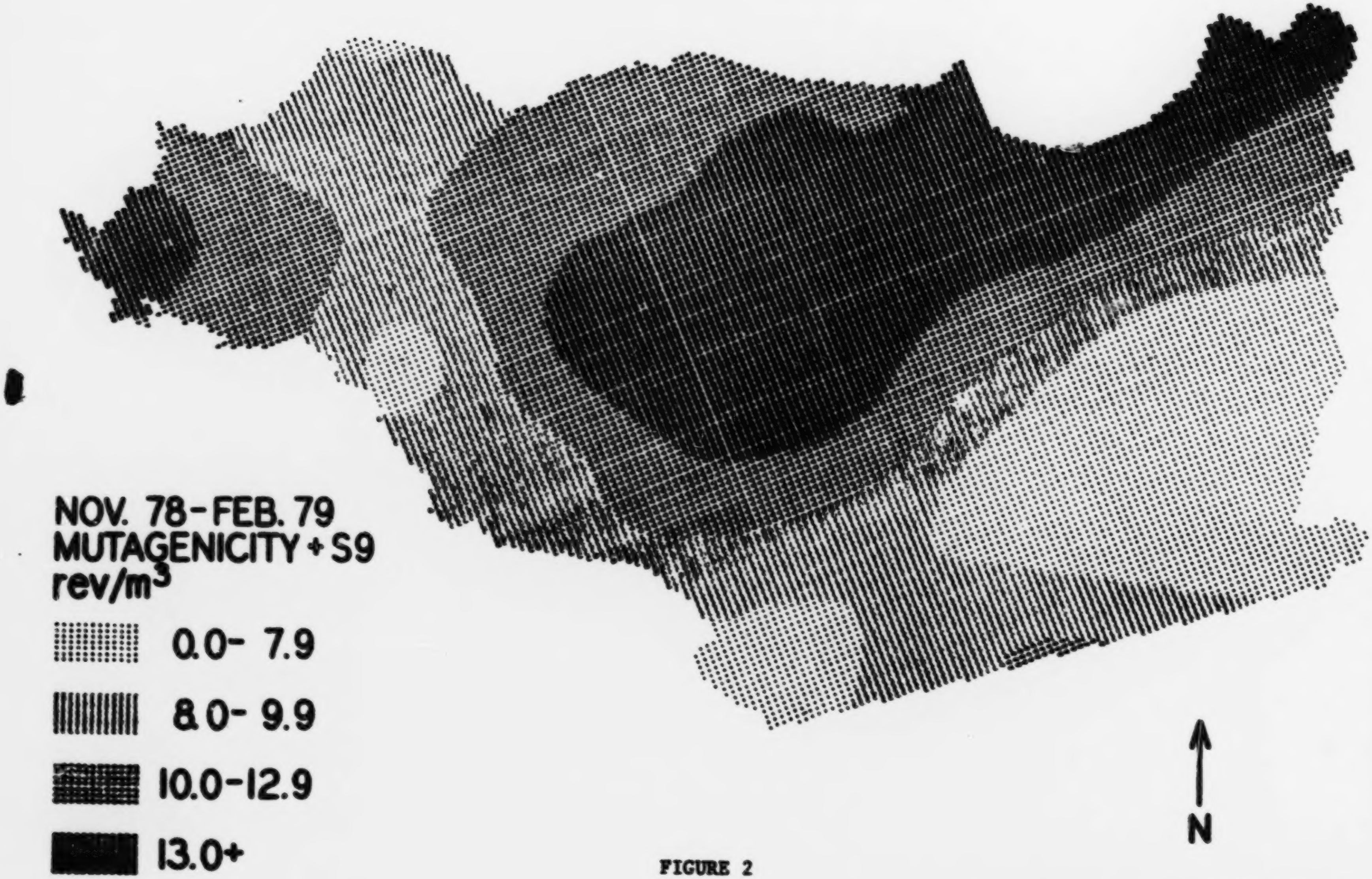

NOV. 78-FEB. 79
MUTAGENICITY + S9
rev/m^3
0.0- 7.9
8.0- 9.9
10.0-12.9
13.0+
N

FIGURE 2

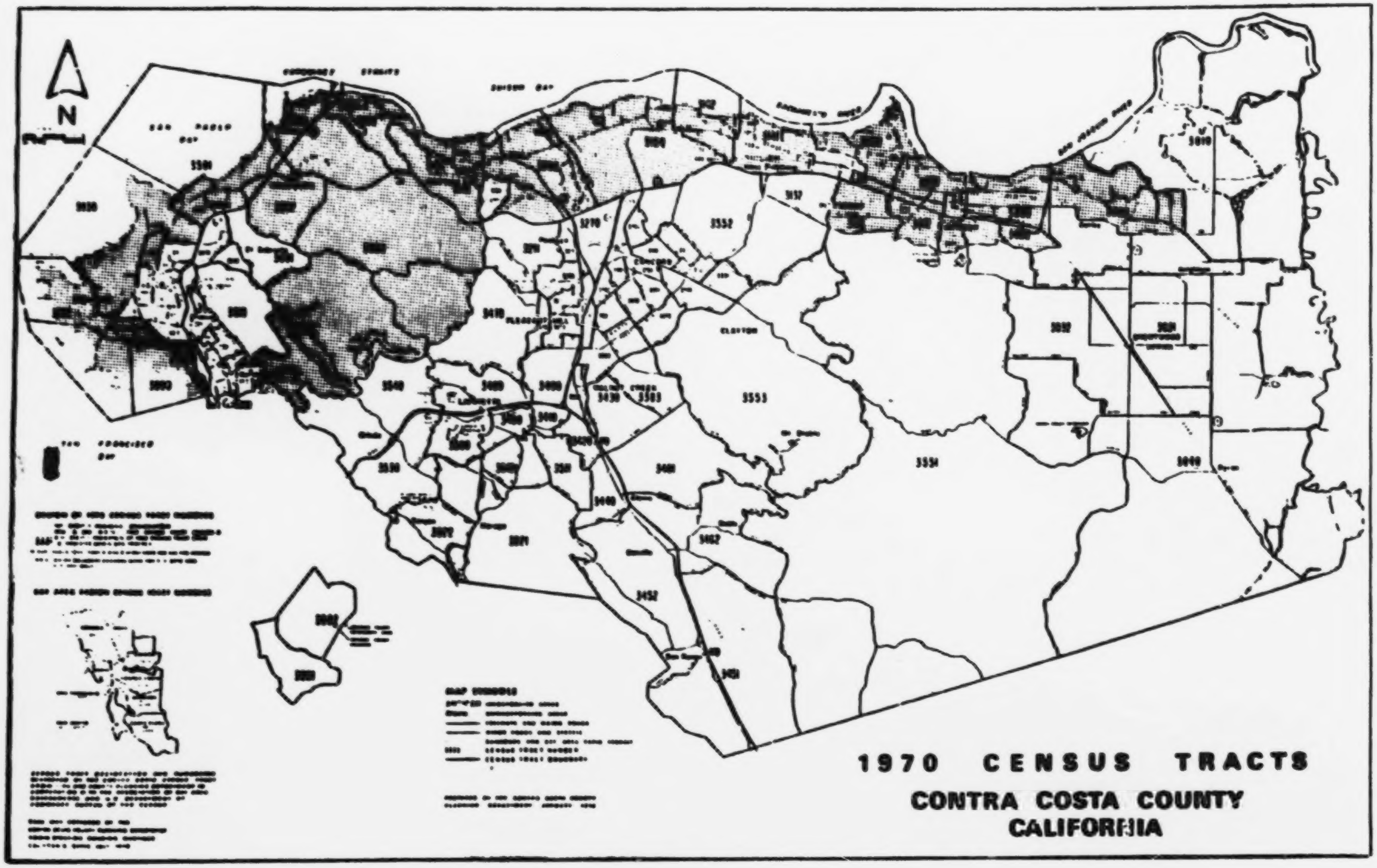

N
SAN PABLO BAY
SUISUN BAY
SAN FRANCISCO BAY
CLAYTON
3552
3553
3554
3157
3452
3451
1970 CENSUS TRACTS
CONTRA COSTA COUNTY
CALIFORNIA

FIGURE 3

PROCEEDINGS OF THE
SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

Thursday Afternoon, September 10

Epidemiological and Statistical Session - continued

Session Chairperson:
Dr. Kenneth Bridbord
National Institute for Occupational Safety and Health

AFTERNOON SESSION

DR. BRIDBORD: I would like to welcome everybody back to our afternoon session, a continuation on epidemiology.

I was asked to make a couple of announcements. One is that tomorrow morning's session, chaired by Dr. Yodaiken, will begin at 8:45 a.m. instead of the scheduled nine o'clock. That is to accommodate Dr. Sober who will present a paper at 8:45 instead of at three o'clock that afternoon.

In addition, there will be a change in the order of the papers for this afternoon's session with the paper by John Patterson from National Center for Health Statistics being at the end of this session.

I would also ask if people at all possible could direct some very specific questions to the authors following their presentations, those questions that would be of immediate interest to them in order to speed us along. But, certainly, if there is an important question or issue that needs to be raised for the entire group, we should hear that.

PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

The Mortality Experience of New York City Newspaper Pressmen, 1950-1976 *

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Irving J. Selikoff

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and

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*This paper was presented by

Frank B. Stern
National Institute for Occupational Safety and Health

The mortality experience of New York City newspaper pressmen, 1950 - 1976

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and Irving J. Selikoff

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Mount Sinai School of Medicine of The
City University of New York
New York, New York
and
American Cancer Society*
New York, New York

ENVIRONMENTAL SCIENCES LABORATORY
MOUNT SINAI SCHOOL OF MEDICINE OF THE CITY UNIVERSITY OF NEW YORK

Introduction

A number of epidemiological investigations have suggested that there may be a cancer hazard associated with newspaper pressroom work in the printing trades. These included an analysis by Moss, Scott, and Atherley[1] of the mortality experience of printing trades workers, 1954-1969, in Manchester and London, which indicated an excess of cancer deaths among these men. For the pressroom workmen, the percentage of excess deaths from cancer of the lung and bronchus was 103% for employees of Manchester papers, and 24% for London newspaper pressmen. In Manchester, 38 of 201 deaths (18.9%) were from cancer of the lung and bronchus. These investigators believed that cigarette smoking was unlikely to entirely explain their findings, since bronchitis and emphysema deaths were not simultaneously increased.

Similar results were obtained in a separate study of London pressmen by the British Department of Productivity and Employment.[2] They found a 20% increase in mortality from cancer and a 33% increase in deaths from bronchogenic carcinomas in all printing workers in a London newspaper. As the firm studied was included in those surveyed by Moss, Scott and Atherley, the study cannot be considered independent. In the United States, an analysis by Lloyd, Decoufle and Salvin[3] of mortality patterns encountered in 676 deaths, 1966-1968, among U.S. newspaper pressmen suggested an increased proportion of cancer deaths, especially of the oropharyngeal area in younger workers. Cancers of the respiratory system (PMR=120), cancers of the digestive system (PMR=121), and leukemia (PMR=151) were also elevated, but not at the 5% level of significance. An excess of deaths from liver cirrhosis (PMR=161), however, was of statistical significance. Similar, but less elevated mortality patterns, were also seen among commercial pressmen.

Each of these studies used proportionate mortality analyses and did not evaluate the experience of defined populations at risk. Thus, the results could only be considered indicative of possible risk because of the inherent limitations of the methodological approach.

A fourth study, by Pasternack and Ehrlich,[4] compared deaths among the pressroom employees of a single newspaper with those of compositors in the same printing plant. The results showed an increased risk of death of pressmen, but only for those first employed after the age of 40. As these individuals would likely have had previous employment in other pressrooms, this suggested that a varying disease response may have been associated with different pressroom exposures. As the study population was relatively small, no specific causes of death were likely to be significantly elevated, nor were any found. An earlier mortality study of the same plant workforce, but with fewer deaths, showed no unusual patterns of death among pressmen compared to compositors.[5]

The results of two cohort studies have been published recently. In one, by Paganini-Hill, Glazer, Henderson, and Ross,[6] 1,361 Los Angeles pressmen were studied from the years 1949-1965 through 1978. Of the 344 deaths for which cause was known, elevated risks of death were found for leukemia (SMR=247), cancer of the kidney (SMR=303), liver cirrhosis (SMR=205) (all with $p < 0.05$), and cancer of the lung and trachea (SMR=149). The study was somewhat limited by the relatively few deaths (354), by the number of untraced individuals (120), and by the fact that 64% of those with known work histories had less than 30 years of pressroom employment. The second study, by Bertazzi and Zocchetti[7] of newspaper workers in Milan, Italy had few pressmen in the group observed (only 23 deaths were available for analysis).

Earlier work, with less substantive data than the above studies, had suggested an increased risk of death of printing trades workers from lung cancer[8] [9] or leukemia.[10] Other data showed no such elevation.[11] In these studies, estimates of risk were made using the listing of occupation on certificates of death and thus were subject to the inaccuracies inherent in that procedure.

As all of the above studies had specific limitations, the desirability of further study of the mortality of workers in the printing trades is evident. We have identified a group of newspaper pressmen, employed in the pressrooms of New York City newspapers, suitable for such an investigation.

Population for study

A cohort was established, composed of all active or retired journeymen members on the rolls of Local 2 of the International Printing Pressmen and Assistants Union (I.P.P.A.U), New York City, as of January 1, 1950. Included were 1,769 men actively employed as pressmen, pensioners, or "beneficiary" members (individuals unemployed at the time because of disability). For each of these men, personal information on date of birth, date of union initiation either in Local 2 or other locals in the International Union, and pressmen employment history were available. Excluded were seven individuals for whom only a dues payment record existed. They were apparently "travellers," who were members of another local union elsewhere and worked temporarily in New York City during 1950. No other records of these individuals were found in any files of the New York City local.

The distributions by age and date of initial employment of the cohort are shown in Table 1. Before 1920, some of these individuals entered the trade through employment in a newspaper shop that was not organized by a local of the I.P.P.A.U. and this may not be reflected on union records. Even knowledge of first employment in an union shop may be incomplete, as Local 2 was chartered only in 1923. Prior to that date, a predecessor organization, Local 25, existed, but information on membership in that organization, available through the International Union or indicated on Local 2 records, may not be fully accurate. Thus, age may be a better indicator of first employment in the trade, considering entry to occur from 16 to 18 years, as was the practice. However, as can be seen from Table 1, these uncertainties with regard to time of first employment are relatively minor compared to the total years of employment for most individuals. Further, as will be seen later, there was little difference in the time course of the mortality experience, whether analyzed according to date since initial employment or according to age. It should be noted that achievement of journeyman status usually followed an apprenticeship of 10 or more years. This, and infrequent admissions into the union during the depression years, resulted in the journeyman rolls largely containing individuals who were first employed prior to 1930, and who were 40 years or older in 1950.

Analysis of the cohort mortality experience

We had available for investigation a cohort of 1,769 journeymen newspaper pressmen established as of January 1, 1950. Virtually all members, on that day, would have achieved at least 15 years from onset of employment; more than 93%, twenty or more years; and 44%, more than 30 years from onset. This cohort was traced through December 31, 1976, and vital status was determined for 98.6% of the individuals. Table 2 lists the status of the cohort and knowledge of vital status at that time. Those individuals untraced were either suspended or withdrew from the union and no data are currently available on their vital status. They were considered alive until lost to follow-up and contributed to the person-years at risk for the appropriate periods. Cause of death information was available for all but 11 deaths. The causes were coded according to the classification of deaths in effect during the relevant calendar period of time.

Table 3 provides data on the person-years of observation for the group by age, years since onset of employment, and periods of observation. The expected numbers of deaths for the cohort, by cause, were calculated using quinquennial age and calendar year specific rates applicable to New York City white males. These rates were averaged for five year calendar periods and multiplied by the applicable person-years of observation for each period. The majority of cohort members resided in New York City, although a significant number made their homes in the suburbs (30% from addresses in 1967). For them, use of New York City rates could result in small overestimate of expected mortality. For comparative purposes, the expected number of deaths were also calculated using U.S. rates although these are likely to understate the appropriate expected numbers of deaths. In addition to difficulties in selecting an appropriate geographical rate, the validity of using general population statistics is affected by the likely existence of a different distribution of employed to unemployed in the various age categories of the cohort compared to the general population. While pensioners are included in the cohort, many individuals 70 years or more of age were currently employed in 1950 and virtually all individuals under the age of 60 were active. Thus, one might expect to see some "healthy worker effect" existing for a number of years in the follow-up of these pressmen. This comes about because a population of individuals currently employed is healthier than a correspondingly aged group in the general population, which includes many who are terminally ill or unable to hold, or to have obtained, a job by virtue of disability, illness, serious injury, or congenital defects.

To test whether the ratio of observed to expected deaths differed significantly from unity, the observed deaths were considered to be the realization of a Poisson variate. Under the null hypothesis, the ratio of observed to expected deaths equals unity and two-tailed probabilities may be calculated.[12] At various levels of statistical significance, 0.05, 0.01, and 0.001, the points for rejecting for the null hypothesis were examined. Statistical significance was established at the lowest of these levels of probability, if any.

Table 4 lists expected and observed deaths by cause, for the full period of observation, January 1, 1950 through December 31, 1976. Overall, an excess of mortality over that expected, either by local or national rates, is observed; 1,232 deaths occurred versus 1,102 expected, according to New York City rates. The important causes of death contributing to this excess were cancer, in excess by 18%; non-infectious respiratory diseases, increased 2.49 times; and cardiovascular disease, which was 5% greater than expected. Among the malignancies, lung cancer and cancer of the pharynx and buccal cavity, particularly, are increased significantly. Deficits of mortality are seen for cancers of the gastrointestinal tract. The 51 deaths of noninfectious respiratory disease was largely concentrated in emphysema (24 cases) and chronic obstructive pulmonary disease (13 cases). Other causes were chronic bronchitis (2 cases) asthma (4 cases), unspecified fibrosis (2 cases) and cor pulmonale (3 cases).

To investigate whether unusual cigarette smoking patterns among pressmen could explain the elevated risk of death from lung cancer and other causes, we calculated what the expected mortality experience would be if the cohort were comprised of 20% more cigarette smokers than was typical of the general population. This was done using data available from the prospective American Cancer Society (ACS) study of one million personsl.[13] First, age specific mortality rates, by cause, were calculated using smoking specific rates from the ACS study during the years 1967-1971, weighted according to the age specific distribution of male smoking habits, which in general was that 68% were current or ex-smokers, 22% had never smoked regularly, and 10% smoked pipe and/or cigars only. Such calculations produced age specific rates, $R_i(\text{ACS})$, for each cause of death. The calculations were repeated, assuming 20% more men were current or ex-cigarette smokers and proportionally fewer were nonsmokers and pipe or cigar smokers, yielding values, $R_i(\text{ACS}+20\%)$. The projected deaths for a pressmen cohort smoking 20% more than the general population were then calculated using age and calendar year specific rates given by:

$$R_{ij}(+20\%) = R_{ij}(\text{NYC}) \times \frac{R_i(\text{ACS}+20\%)}{R_i(\text{ACS})}$$

where $R_{ij}(\text{NYC})$ are the standard age and calendar year rates for the New York City general populations rates. The results of these calculations are shown in Table 5. As can be seen, a hypothetical 20% increase in cigarette smoking among the pressman population cannot account for the excess mortality seen in lung cancer, cancer of the pharynx and buccal cavity, and non-infectious respiratory disease. It could, however, bring cardiovascular deaths to within eleven of those observed, 685.47 versus 694 and deaths from heart disease to virtually that observed, 577.53 versus 581.

It should be noted that, to the extent that work-related conditions contributed to the excess mortality, the exposures in question would be those of decades past as the observed causes of excess death (cancer and chronic respiratory disease) are those known to be associated with long lapsed periods between onset of exposure and evidence of disease. To provide some information on possible changes in mortality with time an analysis was made according to calendar year. Results on the expected and observed deaths, by cause, are shown in Table 6. Proportionately less mortality is seen in the first decade of observation, perhaps reflecting factors contributing to the "healthy worker effect" seen in other populations. The relative risks for all causes of death, cardiovascular disease and all cancer were lower for the 1950-1959 period compared to later years.

Tables 7 and 8 provide information on the expected and observed numbers of deaths according to time since initial employment, and by attained age. As mentioned previously, exact knowledge of first employment in the industry does not exist for all cohort members and age may better reflect employment onset than recorded date of initial employment. The analysis by age, however, shows a pattern little different from that found in the analysis by time from initial employment. In each case a uniform excess of mortality is found in all time or age categories after the first (less than 30 years from initial employment or under 50 years of age).

Table 9 summarizes the results of an analysis by subcohorts defined by decades of initial employment and compared at equal times from first employment. The mortality patterns for all causes of death or death from cancer did not reveal any significant trends. Thus, there is no evidence that the conditions, either in or out of the workplace, which contributed to the observed excess mortality changed, at least during the first four or five decades of this century (the years of entry and initial employment of cohort members in the industry).

Work locations, activities and exposures

Union records do not provide data on locations of employment of individuals, either prior to or during the period of follow-up encompassed by this study. Verbal descriptions by union members of conditions in the several newspapers where they were employed indicate that significantly different exposures could have existed for different cohort members. Thus, information on location of employment over the years would be particularly useful. Unfortunately, this information is not yet available from current publishers and for those companies which have ceased newspaper publication it is unlikely ever to be available. (Many New York City newspapers in which these pressmen were employed are no longer published. They include: The World, the Telegram, the New York Herald Tribune, PM, the Evening Graphic, the Journal-American, the Daily Mirror, various merged papers from the above, and others.)

Few published data exist of the air concentrations of the different pollutants in the work environment of newspaper workers. A study by Lippmann and Goldstein[14] in a New York City pressroom showed that time weighted average ink mist (a mineral oil-carbon black mixture) concentrations were approximately 7-8 mg/m^3 for pressroom employees and about 2 mg/m^3 for reelroom workers. Size fractionation of the aerosol indicated that approximately 80% by mass was non-respirable. Thus, the majority of the mineral oil-carbon black ink mist particles would be deposited in the pharyngeal region and upper respiratory track with only a minor portion carried to the lower respiratory region. Table 10 lists the information available on the average ink mist concentrations measured in the Lippmann and Goldstein study. It should be noted that these concentrations were found to be present after the installation of new, relatively high speed presses utilizing vacuum extraction to control ink mist dissemination, and do not necessarily reflect earlier concentrations. These may have been higher by virtue of poorer control technology, or lower by virtue of slower press operating speeds. Eight measurements taken in the pressroom of a Detroit newspaper were reported in 1962[15] and showed concentrations from 2-16.6 mg/m^3 for ink mist particles of all sizes.

Table 11 provides a comparison of mortality ratios for selected causes in this and three previous studies of newspaper pressmen for which sufficient deaths were available to provide meaningful data. All four showed excess cancer of the lung, trachea and bronchus. Two (of three) showed a highly significant excess of malignancy of the buccal cavity and pharynx. Leukemia was in excess in all four studies for which data were reported and kidney and larynx cancer were elevated in the two which provided data on those sites. The remarkable consistency of elevated lung cancer in all of the major studies of pressmen strongly points to a causal relationship with past pressroom exposures. The agent of concern would appear to be the mineral oil-carbon black ink mist. A wide variety of carcinogenic and non-carcinogenic polycyclic aromatic hydrocarbons have been found to be bound to carbon black.[16] [18] Elution of these, however, is difficult, particularly by body fluids. Further the type and quality of adsorbed material depends on the source of carbon black.[16] Mineral oil, too, has been shown to be carcinogenic[21] [23] or possibly so to the gastronintestinal tract,[24] but here, also, the risk of malignancy appears to be related to the source of the mineral oil. Further, no data demonstrate an excess of lung cancer from mineral oil exposure, as seen in the various pressroom studies.

The elevated mortality ratios for cancer of the buccal cavity and pharynx and, perhaps, of the larynx may also be linked to the ink mist exposure. The elevated risks for leukemia may be related to past uses of benzene for cleaning purposes or as a solvent in gravure processes.[25] The excess leukemia seen in the three studies for which data on its risk was supplied is also substantiated in a study of occupation and leukemia by Viadana and Bross[10] who found a relative risk of 1.9 among printers compared with clerks and in a study by Greene, Hoover, Eck and Fraumeni, Jr.[26] who found elevated leukemia mortality among binders and pressmen of the U.S. Government Printing Office. The unusual pattern of elevated noninfectious respiratory disease seen in this study was not present in the other studies, however, and the origin of the difference is uncertain.

Conclusion

This study of the mortality experience of journeymen New York City pressroom employees over the years from 1950 through 1976 showed an increased risk of death from all causes, with significant excesses observed for bronchogenic carcinoma, cancer of buccal cavity and pharnyx, and noninfectious respiratory disease. Cancer of several other sites (larynx, kidney, bladder, prostate, and leukemia) and cardiovascular disease were also elevated, but not at a level of statistical significance ($P<0.05$). The excess mortality for all causes and all cancers occurred in all groups first employed in the decades from 1900 through 1939 and, to the extent that it is occupationally related, would mostly reflect conditions prevalent in the pressrooms of a large number of New York City newspapers with varying exposure circumstances. Mortality experiences of individuals employed in specific newspapers were not available.

In the analysis of mortality patterns the possibility that some of the excesses seen were attributable to unusual cigarette smoking habits was considered. Our computations indicate that this, however, could not account for the observed patterns of excess death from malignancies or respiratory disease.

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Table 1

Age and year of initial employment of New York City newspaper pressmen, on Jan. 1, 1950

	Year of initial employment				
Age on Jan. 1, 1950	Before 1910	1910-1919	1920-1929	1930+	Total
39 or less			65	86	151
40-49		93	524	55	672
50-59	33	281	192	15	321
60-69	158	91	42	7	298
70-79	81	19	9		109
80 or more	13	3	1		18
Totals	285	487	833	164	1,769

Table 2

Status of cohort of New York City newspaper pressmen on Dec. 31, 1976

Status		Number
Traced alive		513
Employed as pressmen	74	
Retired	439	
Traced deceased		1,232
Cause of death ascertained from certificate of death	1,221	
Cause of death unknown	11	
Lost to follow-up		24
1950 - 1959	13	
1960 - 1969	9	
1970 - 1976	2	
Total		1,769

Table 3

The person-years of observation for 1,769
New York City newspaper pressmen by age, years since
onset of employment, and calendar period.

	Years since onset of employment					Periods of observation		
Age	<20	20-29	30-39	40-49	50+	1950-1959	1960-1969	1970-1976
39 or less	272	149	--	--	--	421	--	--
40-49	275	3266	918	--	--	4066	393	--
50-59	122	1393	6994	1208	--	5926	3469	320
60-69	46	359	2366	6591	898	3603	4403	2154
70-79	7	81	399	1654	2851	1343	1905	1744
80 or more	1	16	44	185	860	263	406	439
Totals	722	5264	10721	9539	4611	15621	10577	4658

Table 4

Observed and expected deaths, by cause, among 1,769 New York City pressmen, 1950-1976

Cause of death	Observed	Expected: N.Y. City Rates[1]	Ratio obs/exp	Expected: U.S. Rates[2]	Ratio obs/exp
All Causes	1,232	1,102.31	1.12***	1,018.61	1.21***
All cancer	263	221.94	1.19**	182.00	1.45***
Lung, bronchus and trachea	72	54.80	1.31*	47.28	1.52**
Buccal cavity and pharynx	17	6.84	2.49**	5.74	2.96***
Larynx	5	4.07	1.23	2.80	1.79
Esophagus	4	6.29	0.64	4.40	0.91
Stomach	13	20.80	0.63	14.22	0.91
Colon and rectum	34	38.96	0.87	25.84	1.32
Pancreas	12	12.47	0.96	10.47	1.15
Kidney	7	4.81	1.46	4.08	1.72
Bladder	14	9.40	1.49	7.31	1.92*
Prostate	25	16.32	1.53	18.37	1.36
Leukemia	10	6.92	1.45	7.07	1.41
Lymphomas	8	9.31	0.86	8.54	0.94
All other cancer	42	30.95	1.36	25.88	1.62**
Cardiovascular disease	696	659.68	1.06	613.75	1.13**
Heart disease	581	556.28	1.04	470.92	1.23***
Cerebrovascular lesions	76	70.77	1.07	102.20	0.74
Respiratory disease	107	72.03	1.48***	69.46	1.54***
Pulmonary tuberculosis	11	11.09	0.99	7.59	1.45
Pneumonia and influenza	45	40.02	1.12	27.38	1.64*
Non-infectious respiratory disease	51	20.92	2.44***	34.49	1.48*
Diabetes	17	17.64	0.96	15.01	1.13
Cirrhosis of liver	31	24.00	1.29	14.03	2.21***
Accidents, suicide and violence	39	38.64	1.01	47.60	0.82
All other causes	79	68.38	1.16	76.76	1.03

* 0.01<P<0.05
** 0.001<P<0.01
*** P<0.001

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[1] For the years 1965-1976, New York City rates were obtained from the U.S. National Center for Health Statistics and, for the years 1950-1964, from the National Cancer Institute for cancer, and from the N.Y. State Office of Vital Statistics for nonmalignant causes.

[2] See text for calculation.

Table 5

Observed deaths, by cause, among 1,769 New York City pressmen compared to expected deaths in a population calculated to have 20% added cigarette smokers, January 1, 1950 - December 31, 1976

Cause of death	Observed	Expected: N.Y. City Rates[1]	Ratio obs/exp	Expected: N.Y. rates +20 Smokers[2]	Ratio obs/exp
All Causes	1,232	1,102.31	1.12***	1,156.55	1.07*
All cancer	263	221.94	1.19**	237.17	1.11
Lung, bronchus and trachea	72	54.80	1.31*	63.75	1.13
Buccal cavity and pharynx	17	6.84	2.49**	7.65	2.22**
Larynx	5	4.07	1.23	4.56	1.10
Esophagus	4	6.29	0.64	6.75	0.59
Stomach	13	20.80	0.63	21.92	0.59
Colon and rectum	34	38.96	0.87	38.96	0.87
Pancreas	12	12.47	0.96	13.25	0.91
Kidney	7	4.81	1.46	5.09	1.38
Bladder	14	9.40	1.49	10.22	1.37
Prostate	25	16.32	1.53	16.60	1.51
Leukemia	10	6.92	1.45	6.95	1.44
Lymphomas	8	9.31	0.86	9.45	0.85
All other cancer	42	30.95	1.36	32.12	1.31
Cardiovascular disease	696	659.68	1.06	685.47	1.02
Heart disease	581	556.28	1.04	577.53	1.01
Cerebrovascular lesions	76	70.77	1.07	72.93	1.04
Respiratory disease	107	72.03	1.48***	47.87	1.37**
Pulmonary tuberculosis	11	11.09	0.99	11.92	0.92
Pneumonia and influenza	45	40.02	1.12	42.31	1.06
Non-infectious respiratory disease	51	20.92	2.44***	23.62	2.16***
Diabetes	17	17.64	0.96	17.55	0.97
Cirrhosis of liver	31	24.00	1.29	25.84	1.20
Accidents, suicide and violence	39	38.64	1.01	39.64	0.98
All other causes	79	68.38	1.16	72.90	0.95

* $0.01<P<0.05$
** $0.001<P<0.01$
*** $P<0.001$

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[1] For the years 1965-1976, New York City rates were obtained from the U.S. National Center for Health Statistics and, for the years 1950-1964, from the National Cancer Institute for cancer, and from the N.Y. State Office of Vital Statistics for nonmalignant causes.

[2] See text for calculation.

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Table 6

Observed deaths and ratios of observed to expected deaths among New York City newspaper pressmen, Jan. 1, 1950 - Dec. 31, 1976, by calendar years

	Calendar year								
	1950-1959			1960-1969			1970-1976		
Cause of death	Obs.	Exp.[1]	O/E	Obs.	Exp.	O/E	Obs.	Exp.	O/E
All causes	436	413.62	1.07	499	428.85	1.16**	297	260.61	1.14**
All cancer	92	81.59	1.13	106	86.25	1.23*	65	54.10	1.20
Lung, bronchus & trachea	22	17.81	1.24	34	22.24	1.53*	16	14.75	1.08
Buccal cavity and pharynx	6	2.78	2.16	4	2.63	...	7	1.43	4.90**
Gastrointestinal	21	24.43	0.86	19	22.34	0.85	7	12.99	0.54
All other cancer	43	36.57	1.18	49	39.04	1.26	35	24.93	1.40
Cardiovascular disease	243	247.60	0.98	286	257.46	1.11	167	154.62	1.08
Heart disease	199	211.35	0.94	241	216.92	1.11	141	128.01	1.10
Cerebrovascular lesions	28	25.71	1.09	31	27.54	1.13	17	17.53	0.97
Non-infectious resp. disease	5	4.43	1.13	22	8.81	2.50***	24	7.68	3.13***
Cirrhosis of liver	15	9.35	1.60	12	10.51	1.14	4	4.14	...
Accidents, suicide & violence	17	18.07	0.94	16	13.64	1.17	6	6.92	0.87
All other causes	64	52.58	1.22	57	52.18	1.09	31	33.15	0.94

* $0.01<P<0.05$

** $0.001<P<0.01$

*** $P<0.001$

[1] New York City death rates were utilized in computing expected number of deaths.

Table 7

Observed deaths and ratios of observed to expected deaths among New York City newspaper pressmen, Jan. 1, 1950 - Dec. 31, 1976, according to time from initial employment

	Years from initial employment											
	<30			30-39			40-49			50+		
Cause of death	Obs.	Exp.[1]	O/E	Obs.	Exp.	O/E	Obs.	Exp.	O/E	Obs.	Exp.	O/E
All causes	63	72.75	0.87	279	241.85	1.15*	462	403.35	1.15**	428	384.36	1.11*
All cancer	17	14.14	1.20	59	51.46	1.15	104	86.17	1.21	83	70.16	1.18
Lung, bronchus, and trachea	3	3.44	...	15	13.66	1.10	34	22.40	1.52*	20	15.30	1.31
Buccal cavity & pharynx	0	0.48	...	5	1.84	2.72	7	2.70	2.59*	5	1.82	2.75
Gastrointestinal	8	3.73	2.14	11	13.27	0.83	17	22.86	0.74	11	19.89	0.55
All other cancer	5	6.49	0.77	28	22.69	1.23	46	38.21	1.20	47	33.15	1.42
Cardiovascular disease	29	39.62	0.73	150	137.19	1.09	259	239.97	1.08	258	242.90	1.06
Heart disease	26	34.52	0.75	124	120.17	1.03	219	203.77	1.07	212	197.82	1.07
Cerebrovascular lesions	2	3.32	...	18	12.03	1.50	22	24.82	0.89	34	30.61	1.11
Non-infectious respiratory	0	0.83	...	6	3.63	1.65	26	8.03	3.24***	19	8.43	2.25***
Cirrhosis of the liver	5	2.83	1.77	16	8.39	1.91*	5	9.13	0.55	5	3.65	1.37
Accidents, suicide & violence	2	4.78	...	11	10.86	1.01	11	12.92	0.85	15	10.08	1.49
All other causes	10	10.55	0.95	37	30.32	1.22	57	47.13	1.21	48	49.14	0.98

* $0.01<P<0.05$
** $0.001<P<0.01$
*** $P<0.001$

[1] New York City death rates were utilized in computing expected number of deaths.

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Table 8

Observed deaths and ratios of observed to expected deaths among New York City newspaper pressmen, Jan. 1, 1950 - Dec. 31, 1976, according to attained age

	Attained age											
	<50			50-59			60-69			70+		
	Obs.	Exp.[1]	O/E	Obs.	Exp.	O/E	Obs.	Exp.	O/E	Obs.	Exp.	O/E
All causes	31	31.64	0.98	200	162.82	1.23**	396	367.45	1.08	605	540.41	1.12**
All cancer	8	5.58	1.43	41	35.01	1.17	91	83.89	1.08	123	97.46	1.26*
Lung, bronchus, and trachea	1	1.32	...	15	9.93	1.51	25	23.54	1.06	31	20.00	1.55*
Buccal cavity & pharynx	0	0.19	...	2	1.38	...	10	2.71	3.69**	5	2.56	1.95
Gastrointestinal	5	1.31	3.82*	9	8.25	1.09	11	21.48	0.51	22	28.71	0.77
All other cancer	2	2.76	...	15	15.45	0.97	46	36.16	1.27	65	46.19	1.41*
Cardiovascular disease	10	15.11	0.66	103	87.99	1.17	224	211.57	1.06	359	345.01	1.04
Heart disease	9	13.36	0.67	89	78.96	1.13	190	183.58	1.03	293	280.38	1.05
Cerebrovascular lesions	1	0.97	...	12	6.32	1.90	16	19.39	0.83	47	44.10	1.07
Non-infectious respiratory	0	0.28	...	2	2.28	0.88	20	7.46	2.68***	29	10.90	2.66***
Cirrhosis of liver	4	1.86	...	13	7.57	1.72	8	9.97	0.80	6	4.60	1.30
Accidents, suicide & violence	3	3.16	...	10	8.85	1.13	7	12.27	0.57	19	14.36	1.32
All other causes	6	5.65	1.06	31	21.12	1.47	46	42.29	1.09	69	68.08	1.01

* 0.01<P<0.05

** 0.001<P<0.01

*** P<0.001

[1] New York City death rates were utilized in computing expected number of deaths.

BEST DOCUMENT AVAILABLE

Table 9

The ratios of observed to expected deaths of New York City pressmen for all causes of death and all cancer deaths, according to date of initial employment and years since initiation, Jan. 1, 1950 - Dec. 31, 1976[1]

	Years since initial employment				
Year of initial employment	<30	30-39	40-49	50+	
Before 1910	--	--	0.77	1.12	all causes
	--	--	0.63	1.40	all cancers
1910 - 1919	—	1.19	1.23	1.06	all causes
	—	1.14	1.24	1.00	all cancers
1920 - 1929	1.03	1.11	1.26	1.27	all causes
	1.42	1.07	1.44	1.02	all cancers
1930+	0.62	1.33	0.60	—	all causes
	0.88	1.59	--	--	all cancers

---

[1] New York City death rates were utilized in computing expected number of deaths.

Table 10

Time-weighted average oil-mist exposures
43rd Street pressroom of the New York Times

Exposed Groups	Work Phase	Time at phase (min)	No. of samples	Average oil-mist concentration (mg/cu m)		
				Large	Small	Total
Goss press, pressmen, and helpers	Production	330	70	10.40	1.80	12.20
	Press makeup and breakdown	120	8	0.22	0.41	0.63
	Rest break	30	2	0.53	0.77	1.30
	Time-weighted average	480		7.24	1.39	8.63
Goss press, reelroom crew	Production	330	18	2.50	0.90	3.40
	Press makeup and breakdown	120	8	0.22	0.41	0.63
	Rest break	30	2	0.53	0.77	1.30
	Time-weighted average	480		1.81	0.77	2.58
Wood press, pressmen and helpers	Production	330	61	8.60	1.20	9.80
	Press makeup and breakdown	120	6	0.17	0.49	0.66
	Rest break	30	2	0.53	0.77	1.30
	Time-weighted average	480		5.98	1.00	6.98
Wood press, reelroom crew	Production	330	24	0.80	0.40	1.20
	Press makeup and breakdown	120	6	0.17	0.49	0.66
	Rest break	30	2	0.53	0.77	1.30
	Time-weighted average	480		0.63	0.44	1.07

From Lippmann and Goldstein (1970).

Table 11

A comparison of mortality ratios for selected causes of death among newspaper pressmen in four mortality studies

Cause of death	This study SMR	Moss et al. PMR	Lloyd et al. PMR	Paganini-Hill et al. SMR
All cancer	118** (262)	ND	111 (138)	98 (68)
Lung and trachea	131* (72)	138** (109)	113 (47)	149 (22)
Buccal cavity and pharynx	249** (17)	ND	237* (9)	92 (2)
Larynx	123 (5)	ND	211 (4)	ND
Kidney	146 (7)	ND	ND	303* (5)
Bladder	149 (14)	ND	ND	85 (2)
Leukemia	145 (10)	ND	157 (8)	247* (7)
Non-malignant respiratory disease	148***(107)	92 (62)[1]	97 (39)	ND
Liver cirrhosis	129 (31)	ND	161 (22)	205* (17)

SMR	Standard mortality ratio.
PMR	Proportionate mortality ratio (to total deaths).
( )	Number of deaths on which mortality ratio is based.
ND	No date available.
1	Chronic bronchitis and emphysema.
*	$0.01 < p < 0.05$
**	$0.001 < p < 0.01$
***	$p < 0.001$

DR. YODAIKEN: I know that the conditions in the pressrooms have changed considerably over the last five years, but do you know of any significant changes that occurred before that time?

DR. STERN: Actually, the environmental study by Littman was conducted in 1970, and it still showed that the ink mist exposures were higher than the permissible standard.

There were environmental samples taken by Mt. Sinai, but I do not have the complete results right now. Based upon Littman's study in 1970, some of these exposures were still relatively high.

PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Environmental Health Data Base for New Jersey

Thomas Burke

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Trenton, NJ 08625

The Office of Cancer and Toxic Substances Research (OCTSR) of the New Jersey Department of Environmental Protection (NJDEP) was established in 1977 as a result of New Jersey's nation leading cancer mortality rates and growing concern about the presence of carcinogens and toxic substances in the environment. New Jersey is the most densely populated, most industrialized state in the nation and petrochemicals are the number one industry. While hypotheses concerning the state's cancer rates and environment abound, the truth is that before 1977 virtually nothing was known concerning the exposure of New Jersey citizens to environmental toxics. The long term goal of OCTSR is to establish a statewide profile of the distribution of toxics and carcinogens in the environment in order to aid in the regulatory and enforcement activities of NJDEP, develop standards for the control of these substances, and ultimately to better understand the relationships between these pollutants and human disease.

During the past four years OCTSR has initiated a number of statewide monitoring, sampling and survey projects to develop a comprehensive approach to the evaluation of population exposure to toxic pollutants. The Environmental Health Database Project was initiated to assist the National Cancer Institute in obtaining environmental exposure data for the New Jersey section of the national case-control study of bladder cancer. The project is enabling OCTSR to construct a computerized database to facilitate rapid statistical, geographic and correlational analysis of environmental and health data. The data consist of five major categories: industrial sources, water quality, hazardous waste, air quality and health data. The following is a summary of ongoing projects and the information planned for inclusion in the database.

1. Industrial Source Information

- Statewide Survey of Industry including information on the production, use, emission and disposal of approximately 200 toxic and carcinogenic substances for 15,000 plants.

  environmental monitoring for sites demonstrating high use and emission;

  listing of permitted water dischargers and plants discharging waste into public sewage treatment plants;

  general air emissions data from air pollution control permit system;

  Ames testing of selected high risk industrial effluents.

2. Water Quality Data

- Drinking Water

  listing of public purveyors including service areas, population served and raw water sources;

  monitoring results for volatile organics, heavy metals and pesticides;

  historic results of monitoring conducted by purveyors;

  current and historic chlorination practices;

  Ames testing results on raw and delivered drinking water.

- Groundwater

  results of statewide sampling of groundwater including all major aquifers and all well types;

  public drinking water supply wells;

  private potable wells;

  industrial and landfill monitoring wells.

- Surface water

  results of statewide monitoring of surface waters and sediments for organics, pesticides and heavy metals;

effluent monitoring results;

Ames testing results on ambient surface waters and effluent samples;

results of statewide fish sampling project for pesticides, heavy metals and PCBs.

3. Hazardous Waste

location and monitoring data from over 400 abandoned hazardous waste dumpsites;

location and permit information on operation of hazardous waste disposal facilities;

location of hazardous spills.

4. Air Quality Information

data from statewide air monitoring network for classical pollutants;

toxic pollutant monitoring data including:

  - volatile organics
  - polynuclear aromatic hydrocarbons
  - metals
  - alkylating agents
  - respirable particulates
  - concurrent Ames testing

data from hazardous substance emergencies collected by mobile monitoring lab;

breathing zone samples and body burden measurements from EPA study of human exposure to toxics.

5. Health Data

county and municipal level mortality data for cancer and other major causes;

cancer morbidity data from New Jersey tumor registry.

- U.S. Census Results

statewide hospital admission and discharge records for all causes.

To facilitate the analysis and interrelating of this data extensive computer graphic and mapping capabilities have been developed. Geographic analysis capabilities are essential to understanding the spatial relationships between environmental pollution and potential point sources, but most importantly such mapping capabilities provide a statewide profile of community exposure levels. Figure 1 through 3 are examples of the types of maps which are being generated. Such maps are providing new insights into the trends and spatial distribution of environmental pollutants. As the data are refined and expanded these techniques will facilitate rapid ecological studies providing a geographic profile of the relationship between environmental and disease trends and providing hypotheses for in depth epidemiological investigations.

Information from the database is in constant use for the regulatory activities or NJDEP. Air and water monitoring results have provided needed baseline information on ubiquitous toxic pollutants. Such information has had numerous applications in regulatory decision making. The drinking water monitoring has resulted in numerous closures of water sources, and is currently being used in the development of guidelines for toxic contaminants. Data from the industrial survey has provided the first real understanding of production, use emission and disposal practices, providing an invaluable regulatory tool.

Monitoring has demonstrated that New Jersey citizens and most likely all Americans are constantly being exposed to toxic

Figure 1

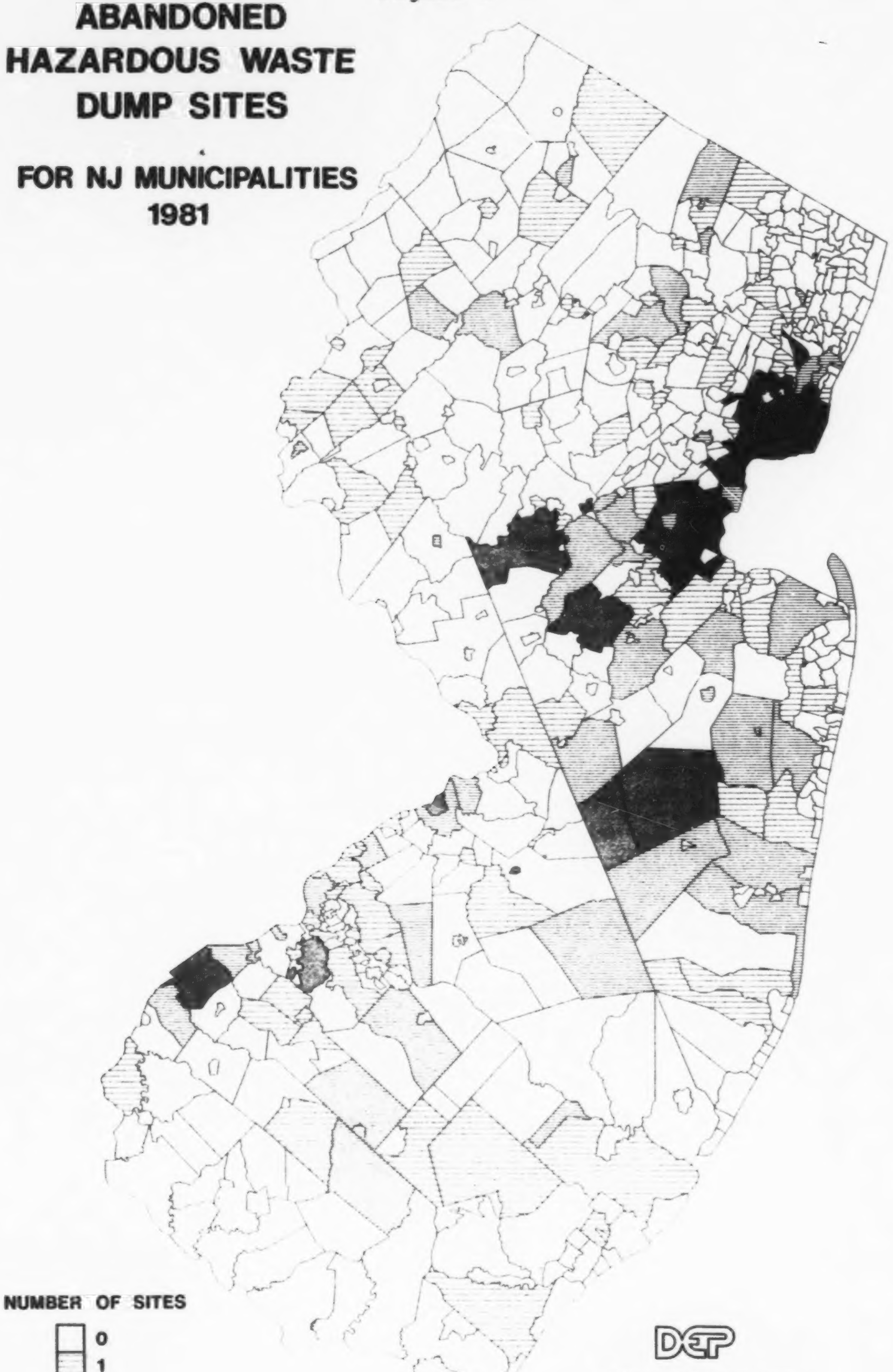
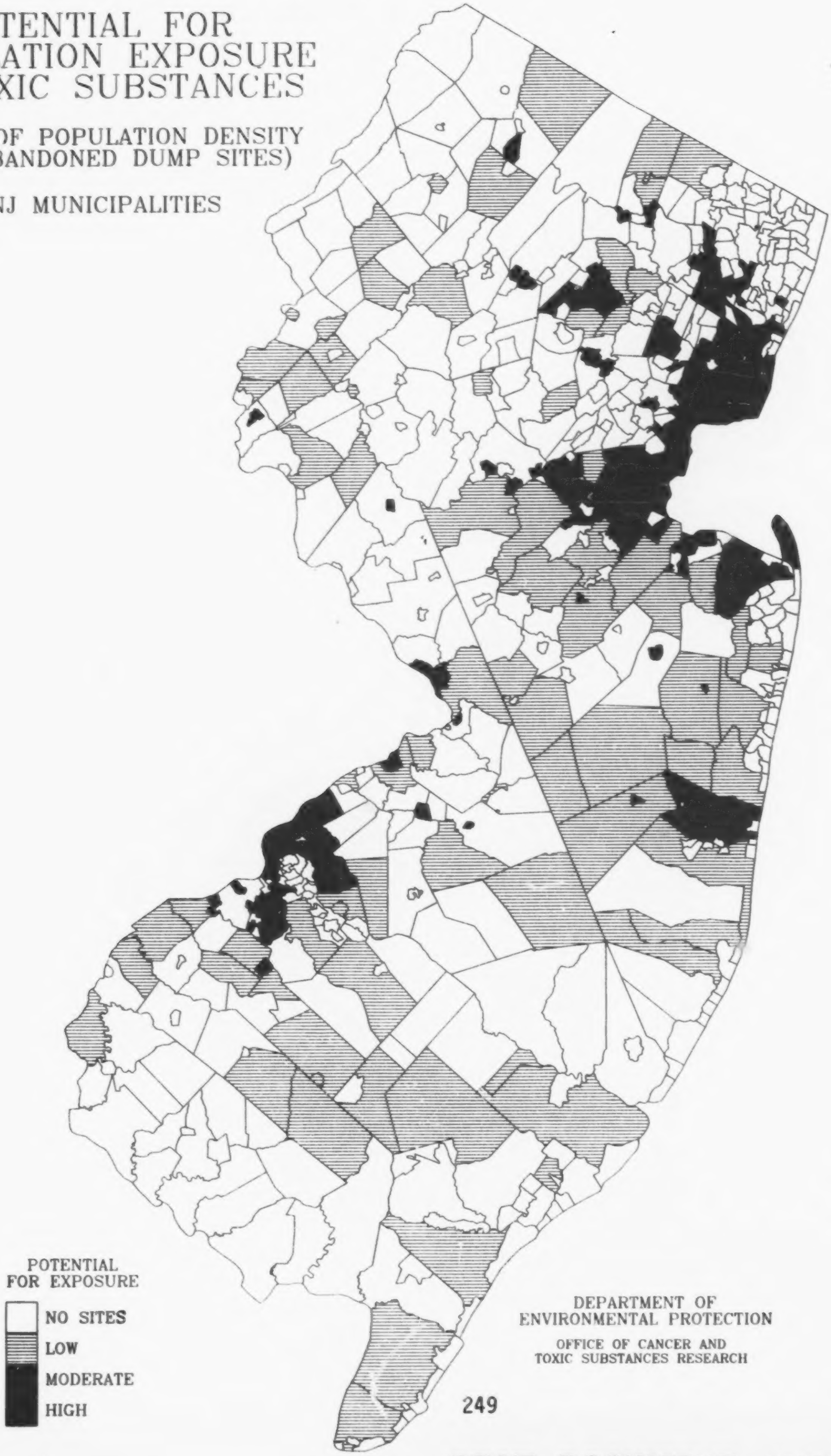

ABANDONED
HAZARDOUS WASTE
DUMP SITES
FOR NJ MUNICIPALITIES
1981
NUMBER OF SITES
0
1
2–3
4–7
8–39
DEP
Office of Cancer and
Toxic Substances Research

Figure 2

POTENTIAL FOR POPULATION EXPOSURE TO TOXIC SUBSTANCES

(INDEX OF POPULATION DENSITY AND ABANDONED DUMP SITES)

NJ MUNICIPALITIES


POTENTIAL FOR EXPOSURE
NO SITES
LOW
MODERATE
HIGH

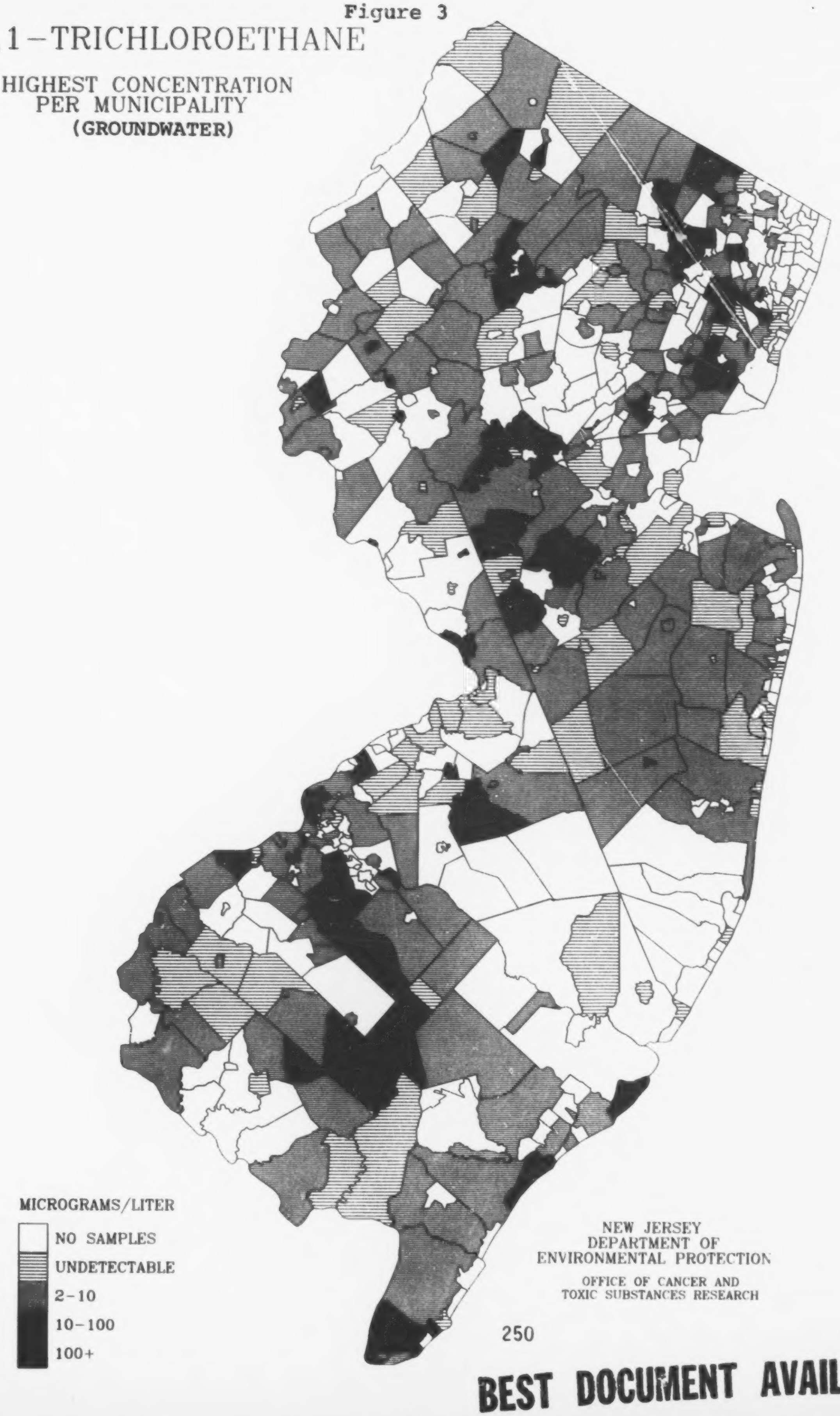
DEPARTMENT OF
ENVIRONMENTAL PROTECTION

OFFICE OF CANCER AND
TOXIC SUBSTANCES RESEARCH

Figure 3

1,1,1-TRICHLOROETHANE

HIGHEST CONCENTRATION
PER MUNICIPALITY
(GROUNDWATER)


MICROGRAMS/LITER
☐ NO SAMPLES
▤ UNDETECTABLE
▩ 2-10
■ 10-100
■ 100+
NEW JERSEY
DEPARTMENT OF
ENVIRONMENTAL PROTECTION
OFFICE OF CANCER AND
TOXIC SUBSTANCES RESEARCH

and carcinogenic agents in the environment. It is hoped that the true value of the database project will be an improved understanding of human health effects. Epidemiologic investigations of the role of environmental pollutants have been largely inconclusive. A major reason has been a scarcity of information on exposure. New Jersey will now have the data resources to clearly define the actual and potential exposure levels of its communities and compare them to disease mortality and morbidity data. This will hopefully stimulate and support future epidemiological investigations and provide new insights to the relationship of the environment to human health.

DR. BEAUMONT: Dr. Beaumont, NIOSH. Are these firms that are currently doing waste disposal, chemical waste disposal, are they actually getting rid of this stuff or are they just saving it for our grandchildren to worry about.

DR. BURKE: My understanding of the problem is that there is a tremendous amount of stockpiling going on throughout the state. New Jersey lacks sufficient disposal facilities. Increased amounts of waste are being burned for energy recovery and in addition, sewage treatment plants are receiving increased amounts of waste. From a public health perspective, current disposal practices are highly inadequate.

DR. CARNOW: Carnow, Illinois. What body burdens -- you mentioned you were measuring body burdens -- what body burdens are you measuring?

DR. BURKE: I am not measuring; R&D from EPA is doing the monitoring. It was a very ambitious list including metals, pesticides and organics to begin with, but when they field tested it on just nine people they realized the list was too ambitious and it has come down to about 15 volatile organics in urine, blood and breath. For a small subset of that, they are testing for metals and pesticides in mothers' milk and blood.

DR. CARNOW: I ask because by examining the metals you might get some insight into petroleum effluents since they are contained in it. And now with some of the atomic absorption units, which can measure 15 or 16 metals simultaneously in a very short time, you can do body burden measurements in a lot of people in a very short time.

DR. BURKE: I am kind of disappointed they are not doing the metals in the whole group.

DR. CARNOW: Well, the metals are easier to measure, you see.

DR. BURKE: I think so.

DR. CARNOW: And they are faster and they can give you a lot of insights.

DR. BURKE: The problem is that the Food and Drug Administration representatives were there in the planning of the study and if you don't consider food intake of metals, which is the major intake -- they decided that measuring metals wasn't very useful because the environmental exposure is only a small percentage of the total.

DR. AUSTIN: Austin from California. Did I understand that you just said that you were stopping the Ames test on ambient air and other environmental --?

DR. BURKE: No. Ames testing has presented a real scientific problem, and that is, what does it mean? I wouldn't say we are stopping it. In fact, we are going full steam ahead with Ames testing of respirable particulates. It has been very fruitful there.

We have major problems in methodology in using the Ames test on drinking water. Essentially, we seem to need a composite sample. The free chlorine in the water from the finished drinking water attacks the resin and alters the sample in such a way that we would not have a representative sample. We can use it in raw water but for drinking water the interpretation has been difficult.

PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Mortality Study of Dry Cleaner Workers Exposed to Perchloroethylene

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and

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Menlo Park, CA

MORTALITY STUDY OF DRY CLEANER WORKERS EXPOSED TO PERCHLOROETHYLENE

I. Introduction

Perchloroethylene (PCE) or tetrachlorethylene is similar in structure to vinyl chloride (Slide 1), a known carcinogen. PCE was tested by NCI for carcinogenicity in 1977, and among rats administered PCE, there was no significant increase in neoplastic lesions. In mice there was a significant increase in the incidence of hepatocellular carcinoma. (NCI, '77)

Because of these results and because of the large number of individuals potentially exposed to PCE, a decision was made by NIOSH to conduct an epidemiologic study to determine whether or not there are any latent effects from long-term exposure to PCE specifically focusing on the carcinogenic risk among exposed individuals. The study was conducted under contract by SRI. Workers in the drycleaning industry were chosen for this study. PCE began replacing carbon tetrachloride in commercial drycleaning shops shortly after WW II. A gradual shift from petroleum derivatives to PCE began in the late 1940's - early 1950's and by 1977 the industry estimated that 74% of commercial drycleaning plants used PCE. However, in the period before 1960, petroleum derivatives was the predominant solvent.

II. Methods

A. Definition of the Study Cohort and Study Design (Slide 2)

The intent of this study was to conduct a retrospective cohort mortality analysis on at least 2000 drycleaner workers who were employed for at least 1 year before 1960 in a drycleaning facility where the primary solvent was PCE. In addition, if the worker had previous occupational exposure to carbon tetrachloride, he/she was not eligible for the study. In this type of analysis the identified cohort is followed-up to determine who is alive and who is dead as of a certain cutoff date. The expected mortality of the cohort is then calculated based on the number of person-years at risk of dying multiplied by the appropriate age, race, sex, calendar time, and specific U.S. mortality rates. The observed cause specific deaths are compared to those expected yielding a measure of risk known as the standardized mortality ratio (SMR = observed deaths/expected deaths x 100). The SMR is evaluated to determine whether or not the cohort has experienced any unusual patterns of death that may be related to occupational exposure. Other variables examined to assess this relationship include latency (length of time since first exposure or employment) and duration of employment.

B. Potential Data Sources Considered for Study

A number of different data sources were examined by SRI in an effort to identify a suitable cohort of drycleaner workers exposed to PCE (Slide 3).

1. Direct identification of individuals through state licensing systems.

   Records were not kept past five years.

2. Identification of drycleaning establishments from:

   - trade associations
   - government licensing authorities
   - manufacturers of drycleaning equipment

   Shops could be identified; however, individual shops did not retain records to identify employees.

3. Ohio State Prison

   Records were destroyed in a fire.

4. U.S. Navy

Pre-1960 records destroyed after computerization of system.

5. Unions

Five large labor unions were found to have records that identified individual employees and the shop they worked for.

- o Oakland local AFL-CIO Laundry and Dry Cleaning Int'l
- o Chicago and St. Louis locals Laundry, Dry Cleaning, and Dyehouse Workers' Int'l
- o Detroit and New York City locals Amalgamated Clothing and Textile Workers Union

Since the St. Louis local was already being used by NCI in a similar study, this union was not considered by SRI. The records from the remaining four union locals were selected for inclusion into the study.

C. Solvent History

To determine whether a particular union member qualified for the study it was necessary to obtain the history of solvent use for each shop the workers were employed at. A list of these shops was assembled and an attempt was made to find out the solvent history for each by using the following data sources: (Slide 4)

1. Contacting present owners and past owners

2. Union officials

3. Local distributors of drycleaning equipment

4. Local distributors of perchloroethylene

5. City records in Chicago

6. Trade associations

7. Licensing authorities

There has been three primary solvents of concern in drycleaning -- (1) Carbon tetrachloride which was used rarely after WW II; (2) Petroleum derivates which were used extensively prior to 1960;

and, (3) PCE which became the predominant solvent after 1960. Based on available information, if it could be documented that a worker was employed for at least one year prior to 1960 in a shop where PCE was the primary solvent, he/she was included in the study as long as he/she had no previous employment in a shop where carbon tetrachloride was the primary solvent. Unknown solvent use prior to 1960 was probably PCE or petroleum derivates. If a worker qualified for inclusion in the study based on work in a PCE shop, time spent in a shop of unknown solvent use did not disqualify the worker from the study, however, this time was not included in calculating the length of employment or latency variables. In addition, the workers were not considered at risk until they accumulated one year of employment in a PCE shop. Since solvent history was not obtained for every shop listed in the union records, many union members who may have been qualified for the study were not included.

D. Vital Status Follow-Up

The follow-up to determine whether workers included in the cohort were alive or deceased was carried out by searching records of the Social Security Administration (SSA), the Internal Revenue Service (IRS), state Motor Vehicle Departments, and the local unions. The cutoff date for vital status determination was set to be September 30, 1977, which was the latest date for which the SSA had vital status information.

Those who died after the cutoff date were considered alive for purposes of analysis. Those whose vital status was unknown were considered alive until lost to follow-up. For those where SSA had indicated the worker had died, the death certificates (DC's) were requested from the State Vital Statistics Offices. Each DC was coded by a trained nosologist according to the revision of the International Classification of Deaths (adapted) ICDA, in effect at the time of death.

III. Industrial Hygiene Study

NIOSH conducted an industrial hygiene survey at drycleaning facilities currently using PCE, some of which were also included in the mortality study (Slide 5). The results as summarized in Table 1 show that the "cleaners" have the highest exposure where the geometric mean for the time weighted average was 22 ppm for machine operators. For all other jobs the highest corresponding value was 3.3 ppm. According to owners and employees interviewed during the survey, the current exposures are not dramatically different from those 20 years ago. From the results of this survey it is evident that exposures experienced by most workers in the study were probably below 20 ppm and many were below 5 ppm.

IV. Results and Discussion

A. Description of Cohort

The total number of workers included in the study consists of 1,597 individuals, 571 males, and 1,026 females. Table 2 (Slide 6) shows the distribution of vital status for males and females, and Table 3 (Slides 8-11) show the same distribution by local union. The success of the follow-up was poor especially for female workers. For this reason, the analysis accumulates person-years for those with unknown vital status only until they are lost to follow-up.

Death certificates on all but 38 of the 285 cohort members known to be deceased were obtained. In calculating the SMR those deaths for which no certificates were found were assumed to have the same distribution by cause as those for which death certificates were available.

The union records did not record race, therefore, an assumption had to be made concerning the racial distribution of the cohort. If the racial distribution from the deceased cohort members can be taken to represent the racial distribution of the entire cohort, approximately 30% of the cohort was black and the remainder was white. The distribution varies depending on the local union. In the analysis the expected number of deaths were calculated by using U.S. white mortality rates (male and female), U.S. black mortality rates (male and female), and by estimating the expected deaths based on the distribution of person-years of those with a known race -- the person-years distribution by age was calculated separately for whites and for blacks. For each five-year age group, the ratio of known white person-years to known black person-years was assumed to represent the racial makeup for the entire cohort within that age group category. White mortality rates were used for the proportion of person-years in each age group that were white, and black rates were used for the remaining person-years to give a new estimate of the expected number of deaths. Because there is some uncertainty in this estimate the emphasis in the discussion of specific causes of death is on those that consistently show an increase regardless of which rates were used.

B. Cause Specific Mortality

The next two tables, 4-5 (Slides 11-12) show the mortality of the entire study cohort compared to the expected mortality. To repeat, the measure of risk is the SMR where an SMR of 100 means the study population has a mortality rate equivalent to that expected based on U.S. mortality rates. The SMR's reported in the text will be based on the estimated expected number of deaths. For all causes, this cohort has a slight deficit in mortality, probably reflecting the healthy worker effect. Among the major categories of death there are no statistically significant increases in observed deaths compared to expected deaths. However, there is a statistically significant deficit in deaths due to accidents, and deaths due to circulatory disease before adjustments were made for unknown deaths. The large deficit in circulatory disease is probably due to both the health worker effect and underascertainment of deaths. There is a slight excess in death, due to all cancer.

When the cancer mortality is examined by specific site, cancer of the intestine except rectum has the highest measure of risk with 11 observed cases, the estimated SMR is 182 or an 82% increase over the expected number of deaths. All 11 observed deaths were from cancer of the colon rather than cancer of the small intestine. Of course, virtually all the expected deaths are also due to cancer of the colon. A more detailed analysis of this cause was undertaken to determine if it was associated with employment in drycleaning. The increased risk was consistent in both males (SMR = 191) and females (SMR = 175) (Slide 13), as well as across all local unions (Slide 14).

When mortality due to colon cancer is examined by latency (Slide 15), no deaths occurred until at least 15 years after initial known exposure to PCE and the risk increases peaking at 20-25 years of latency, a trend compatible with a risk due to an occupational exposure. The risk of colon cancer was also highest for those with ten or more years of employment in PCE shops, a finding also compatible with the risk being associated with employment in drycleaning.

Pancreatic cancer was also looked at in more detail (Slide 16). Although there are only 5 observed deaths, the SMR increases with an increase in the latency period. The increased risk was confined to those with at least 20 years of latency.

V. Conclusions and Recommendations

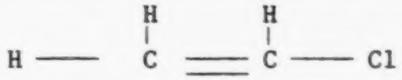
Because of small numbers and several deficiencies (assumed distribution of race and poor follow-up) in this study, it is inappropriate to make any definitive conclusions about the risk of mortality in relation to exposure to PCE. However, the finding of greatest concern is the risk for cancer of the colon, which, based on 11 observed deaths, is consistently elevated across both sexes, all four unions, and the distribution by latency and duration of employment is consistent with an occupationally related disease.

This study is being updated and additional data is being added to the file so that the analysis will require fewer assumptions. This will include (Slide 17): (1) Updating the vital status to 12/31/78; (2) Improving the follow-up of the cohort; (3) Adding data on race; (4) Adding an additional 400 workers to the cohort who were previously left out because date of birth was unknown.

Hopefully, these improvements to the file will yield a study with more valid results and more confident conclusions. Besides colon cancer, pancreatic cancer, genitourinary cancer, as well as diseases of the blood and blood forming organs, will be examined closely.

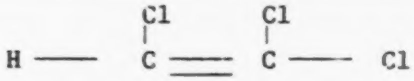
Slide 1

CHEMICAL STRUCTURES

1. 

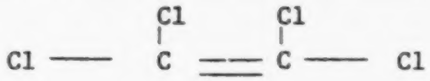
$\mathrm{H-\overset{H}{\overset{|}{C}}=\overset{H}{\overset{|}{C}}-Cl}$

Vinyl Chloride

2. 

$\mathrm{H-\overset{Cl}{\overset{|}{C}}=\overset{Cl}{\overset{|}{C}}-Cl}$

Trichloroethylene

3. 

$\mathrm{Cl-\overset{Cl}{\overset{|}{C}}=\overset{Cl}{\overset{|}{C}}-Cl}$

Tetrachloroethylene (Perchloroethylene-PCE)

Slide 2

DEFINITION OF STUDY COHORT

Each cohort member must have worked for at least one year prior to 1960 in a dry cleaning facility where PCE was the primary solvent during that year, and had no occupational exposure to carbon tetrachloride or trichloroethylene.

Slide 4

POTENTIAL DATA SOURCES TO IDENTIFY COHORT

1. Direct identification of individuals through state licensing systems.
2. Identification of dry cleaning establishments.
3. Ohio State Prison
4. U.S. Navy
5. Unions
    - o Oakland Local AFL-CIO Laundry and Drycleaning International
    - o Chicago and St. Louis Locals Laundry, Drycleaning and Dyehouse Workers International
    - o Detroit and New York City Locals Amalgamated Clothing and Textile Workers Union

Slide 5

SOURCES TO IDENTIFY SOLVENT HISTORY

1. Contacting present and past owners
2. Union officials
3. Local distributors of dry cleaning equipment
4. Local distributors of perchloroethylene
5. City records in Chicago
6. Trade associations
7. Licensing authorities

Slide 6

Table 1

SUMMARY RESULTS OF NIOSH INDUSTRIAL HYGIENE SURVEY - TWA EXPOSURES FROM ALL SURVEYS

Job or Sample Description	Number of Facilities/Samples	Concentration of PCE, ppm		
		Range	Geometric Mean	95% Confidence Interval for Geometric Mean
Dry Cleaner	44/44	4 -149	22	17 - 28
Presser	35/35	0.1- 37	3	2 - 5
Seamstress	12/12	0.6- 29	3	1 - 7
Counter Area	31/31	0.3- 26	3	2 - 5
5 Min. Peak*	39/134	2 -637	49	39 - 61
15 Min. Peak*	30/49	1 -775	34	24 - 50

* The peak samples included clothing transfer.

Slide 7

Table 2

VITAL STATUS BY SEX OF DRY CLEANER WORKERS
EXPOSED TO PCE

	Alive (%)	Dead (%)	Unknown (%)	Total
Males	363 (63.5)	163 (28.5)	45 (7.9)	571
Females	695 (67.7)	122 (11.9)	209 (20.4)	1022
Total	1058 (66.2)	285 (17.8)	254 (15.9)	1597

Slide 8

Table 3

VITAL STATUS BY SEX OF DRY CLEANER WORKERS FROM THE OAKLAND LOCAL UNION

	Alive	Dead	Unknown %	Total
Males	39	14	1 (1.9)	54
Females	62	9	1 (1.4)	72
Total	101	23	2 (1.6)	126

Slide 9

Table 3 (continued)

VITAL STATUS BY SEX OF DRY CLEANER WORKERS FROM THE DETROIT LOCAL UNION

	Alive	Dead	Unknown %	Total
Males	131	81	26 (10.9)	238
Females	263	49	72 (18.8)	384
Total	394	130	98 (15.8)	622

Slide 10

Table 3 (continued)

VITAL STATUS BY SEX OF DRY CLEANER WORKERS FROM THE CHICAGO UNION

	Alive	Dead	Unknown %	Total
Males	58	32	10 (10.0)	100
Females	109	21	38 (22.6)	168
Total	167	53	48 (17.9)	268

Slide 11

Table 3 (continued)

VITAL STATUS BY SEX OF DRY CLEANER WORKERS FROM THE NEW YORK CITY LOCAL

	Alive	Dead	Unknown %	Total
Males	135	36	8 (4.5)	179
Females	261	43	98 (24.3)	402
Total	396	79	106 (18.2)	622

Slide 12

Table 4

MORTALITY AMONG DRY CLEANER WORKERS EXPOSED TO PCE
BY MAJOR CAUSES OF DEATH

Cause	Observed Deaths	Expected Deaths White Rates	Expected Deaths Black Rates	SMR*
All Deaths	285	284.7	473.6	93
All Cancers	73	69.5	86.9	112
Diseases of Blood and Blood Forming Organ	3	0.9	1.5	290
Diseases of Nervous System	26	24.8	59.3	86
Diseases of Circulatory System	87**	124.2	176.8	71**
Diseases of Respiratory System	14	14.5	21.6	93
Diseases of Stomach & Duodenum	4	2.0	2.4	211
Diseases of Genitourinary System	6	3.7	12.7	106
Accidents	1	14.0	21.9	7**
Violence	5	6.5	12.3	65

*Based on estimated distribution of race + adjustment for unknown deaths.

**Difference between observed and expected is significant at $p < 0.01$

Slide 13

Table 5

MORTALITY AMONG DRY CLEANER WORKERS EXPOSED TO PCE BY SPECIFIC CANCER SITE

Cause	Observed Deaths	Expected Deaths White Rates	Expected Deaths Black Rates	SMR*
MN of Intestine (except rectum)	11	7.0	6.8	182
MN of Rectum	3	2.1	2.2	158
MN of Pancreas	5	3.5	4.5	152
MN of Respiratory System	19	14.8	16.5	140
MN of Male Genital Organs	2	2.3	4.9	79
MN of Urinary Organs	5	2.8	2.9	198
MN of Other & Unspecified Sites	11	7.8	8.8	156
Neoplasms of Lymphatic & Hematopoietic Tissues	4	6.0	5.6	77

* Based on estimated distribution of race and adjustment for unknown deaths.

Slide 14

Table 6

MORTALITY FROM CANCER OF THE INTESTINE (EXCEPT RECTUM) BY SEX AMONG DRY CLEANER WORKERS EXPOSED TO PCE

Sex	Observed Deaths	Expected Deaths		SMR*
		White Rates	Black Rates	
Males	5	3.1	2.8	191
Females	6	3.9	4.0	175
Total	11	7	6.8	182

* Estimated

Slide 15

Table 7

MORTALITY FROM CANCER OF THE INTESTINE (EXCEPT RECTUM) BY UNION LOCAL AMONG DRY CLEANER WORKERS EXPOSED TO PCE

Union	Observed Deaths	Expected Deaths		SMR*
		White Rates	Black Rates	
Oakland	2	0.8	0.8	287
Detroit	4	3.4	3.2	130
Chicago	3	1.2	1.1	320
New York City	2	1.7	1.7	146

* Estimated

Slide 16

Table 8

MORTALITY FROM CANCER OF THE INTESTINE (EXCEPT RECTUM) BY LATENCY AMONG DRY CLEANER WORKERS EXPOSED TO PCE

Years of Latency	Observed Deaths	Expected Deaths		SMR's*	
		White Rates	Black Rates	White Rates	Black Rates
15	0	2.89	2.77	--	--
15 20	3	1.83	1.80	189	192
20 25	5	1.39	1.34	415	430
25	2	0.88	0.83	262	289

* Estimated

BEST DOCUMENT AVAILABLE

Slide 17

Table 9

MORTALITY FROM PANCREAS CANCER BY LATENCY
AMONG DRY CLEANER WORKERS EXPOSED TO PCE

Years of Latency	Observed Deaths	Expected Deaths		SMR's	
		White Rates	Black Rates	White Rates	Black Rates
< 20	1	2.30	3.07	50	38
20 - < 25	2	0.71	0.91	325	254
> 25	2	0.44	0.55	524	420

FUTURE ACTIVITIES TO IMPROVE PCE STUDY

1. Updating Vital Status to 12/31/78

2. Improving the Vital Status Follow-Up

3. Adding Data on Race for Each Individual

4. Adding an Additional 400 Workers to Cohort

DR. WEISBURGER: This is the second time I have heard this paper, and for the second time I say, NCI did not select perchloroethylene for test because of the structural similarity to vinyl chloride. The study was conceived, the idea was thought of, long before vinyl chloride was shown to be a carcinogen. It was selected for study because of exposure to lots of people.

DR. BROWN: I stand corrected.

DR. YODAIKEN. Yodaiken, NIOSH. I just wondered, did you do a best case/worst case calculation assuming that all the people who were lost have, in fact, survived?

DR. BROWN: No, we didn't.

DR. INFANTE: On your deaths from blood malignancies, you said your SMR was 290. I couldn't see from where I was sitting how many of those were leukemias and how many were lymphomas. Did you analyze separately or did you combine them?

DR. BROWN: We analyzed separately but I would have to look at my paper to get the figures.

DR. INFANTE: Well, the reason I ask is because the NCI study of dry cleaners shows an excess of leukemia and, they mention in that paper that spotting was done with benzene.

DR. BROWN: Yes.

DR. INFANTE: Now, do you have any information on what spotting was done with in your cohort? And then maybe you ought to look at it to see if you have some leukemias and what cell types you might have.

DR. BROWN: Yes, so many shops were included in this study -- I don't know the number of shops but there is a large number. We don't have information on each individual shop as to what they were using for spotting. They use anything that works on a particular day on that particular stain. Benzene is one of those things.

DR. INFANTE: Then on the pancreatic cancers, you have small numbers but it looks like you have an increase with an increase in latency.

DR. BROWN: Yes.

DR. INFANTE: Did you have autopsy reports on all of those? And what is the possibility that some of those pancreatic cancers might actually have been liver cancers? How much information do you have on the histopathologic diagnosis for the pancreatic cancers?

DR. BROWN: At this point we haven't sent for hospital reports or autopsy reports in those particular cases.

DR. INFANTE: How about liver? I couldn't see from where I was sitting whether you showed liver or not.

DR. BROWN: We didn't find any liver cancers.

MR. FISHER: Bill Fisher, International Fabricare Institute. We are a dry cleaning trade association. Just a comment on what I would have to term a canard about benzene, I doubt that one-half of one percent of the dry cleaning plants even 40 years ago used benzene on a very sporadic basis. Certainly I can tell you that 40 years ago even all of the dry cleaning associations were recommending that it not be used. And there were only very limited cases in which someone would have chosen it. So we don't believe that there really ever has been any exposure to benzene within the industry.

PROCEEDINGS OF THE
SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Chemicals Identified in Human Biological Media

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CHEMICALS IDENTIFIED IN HUMAN BIOLOGICAL MEDIA

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PAPER

Proceedings, EPA/NCI 2nd Annual
Collaborative Workshop on
Environmental and Occupational
Cancer Studies, Rockville,
Maryland, September 9-11, 1981

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*Operated by Union Carbide Corporation under Contract No. W-7405-eng-26 with the U.S. Department of Energy.

CHEMICALS IDENTIFIED IN HUMAN BIOLOGICAL MEDIA

M. Virginia Cone

Chemical Effects Information Center
Information Center Complex
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Oak Ridge, Tennessee 37830

and

Cindy Stroup

U.S. Environmental Protection Agency

The program *Chemicals Identified in Human Biological Media* was inspired by concerns over the need for a centralized source of human body-burden data. Historically, this information, which is needed by research and regulatory agencies involved in the protection of human health, has not been easy to retrieve. The development of this human body-burden data under the direction of Cindy Stroup, U.S. Environmental Protection Agency (EPA), and Herman Kraybill, National Cancer Institute (NCI), has been supported through the NCI/EPA Collaborative Program. Through an interagency agreement between EPA and the U.S. Department of Energy (DOE), the preparation, maintenance, and distribution of the file are being done by the Chemical Effects Information Center in the Information Center Complex, Information Division, at Oak Ridge National Laboratory.

I am glad to have this opportunity to tell you of our recent achievements in this program. I think it will be useful for me to review the scope and structure of the file and, during this discussion, to highlight some unique features and new developments.

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*Operated by Union Carbide Corporation under Contract No. W-7405-eng-26 with the U.S. Department of Energy.

Sources of data for *Chemicals Identified in Human Biological Media* are from the world literature, retrospective to 1974. Manual searches for current literature are routinely done in approximately 60 periodicals. Articles with appropriate data are selected for inclusion in the data base. We have collected nearly 3800 documents; information from approximately 1500 of these documents is in the data base at the present time.

The documents that are selected contain data on body burdens of various chemicals that have been measured in human tissues and body fluids. The data base contains information on 750 chemicals. Approximately 250 of these were added since this time last year. At the time of the last publication, approximately 10% of the total documents in the file concerned pesticides; 30%, drugs; and 40%, metals. The remaining 20% were about other substances, including industrial chemicals and organics which are of interest to NCI and EPA.

Let us look specifically at how the data which we extract are incorporated into the data base and, ultimately, in the published version. The data included are bibliographic, numeric, and textual. Many large files, such as Chemical Abstracts and Biological Abstracts, contain primarily bibliographic information and abstracts. There are approximately 30 data elements, or "fields." Each field contains specific information from the source document. There is general information on language, type of publication, and identification numbers as well as bibliographic and chemical data.

In the past year, we have completed a computerized file of information on all the chemicals in the data base. This file, which contains CAS preferred names, registry numbers, synonyms, formulae, and chemical properties with code numbers specific for each chemical, allows accurate and rapid input of this information into the data base. In addition, the data base contains tissue levels (mean, range), protocols, analysis, number of cases, and information on toxicity, pathology, and health.

In January, our second annual report was distributed to over 800 people and institutions. It contains more than 1900 records, which are displayed in a computer-generated tabular format. One feature of this publication is that it provides the user with various ways to search for information. Records dealing with a specific chemical can be located in various ways by using different listings and directories provided in the document. In addition, there are author, corporate authority, tissue, and keyword indices.

When we began work on this data base, we hoped that ultimately it would be available to on-line users. In May of this year, the data base became available on-line to RECON users. RECON is DOE's computerized information retrieval system, which provides remote terminal access to a variety of data bases. This system, originally developed for NASA by Lockheed, was purchased by the Atomic Energy Commission in the late 1960's (for Nuclear Science Abstracts) and was based at Oak Ridge. Dial-up access began as a regular service in 1976. The system is used by technical libraries, information centers, scientists, and engineers — in all over 600 users. We are working with the National Library of Medicine on plans for having the file on-line there. There are also plans to incorporate some of the information into the Toxicology Data Base at the National Library of Medicine. A tape has been made available to the Commission of the European Communities, which plans to integrate our material in its Environmental Chemicals Data and Information Network. The file will soon be available from Lockheed's DIALOG Information Retrieval Service. This commercial information service has agreed to put the data base in their system at no cost to us.

Levels of chemicals in feral and food animal populations are indicators of both environmental and subsequent human exposures. This concern has prompted the development of a companion data base, *Chemicals Identified in Feral and Food Animals*. Approximately 45 periodicals have been identified as appropriate sources of data. Data elements and tabular display are essentially identical to those used in the human file. We have 300 documents on hand and have begun extractions. We will publish an annual report on some of this material for 1981.

In July, questionnaires were sent to the people who have received the annual publications of the data base. In addition to updating the mailing list, we hoped to learn how many people have access to and use the document, how it is being used, and, most important, how the document can better suit the users' needs. We have not finished compiling the results of this survey, but I would like to share with you what we have learned so far. Over one-third of the questionnaires have been returned, and the comments have been overwhelmingly positive. In most cases, the document is "housed" in private offices and libraries. Over 24,000 people have access to the document. It has had various uses in risk and exposure assessment and as a valuable tool in research and academia. The users' comments have been most gratifying to all of us involved with the project — the document *is* being widely used and is meeting the users' needs.

In addition, many users indicated that they will use the on-line version of the data base. Several pointed out the omission of their favorite chemical, drug, or tissue — in many of these cases, the particular substances have already been included for the next publication. We are always willing to add pertinent information that is brought to our attention. For example, next year's (1982) publication will include data from a new source, the National Environmental Specimen Bank. In addition, we are continually inputting data from the documents we have on hand, covering 1974 to the present time.

DR. LONGFELLOW: I was wondering whether or not your data base would benefit from some editing from the standpoint of setting up certain criteria, let's say, only entering data that, in fact, had been published in reviewed journals, or only entering data that met certain criteria such as a meaningful combination of analytical procedures used. Only using data which provides sufficient information for the reader to arrive at a conclusion independently of the author. I'm suggesting a standardization similar to the prescreening used by the EPA's committees for the Evaluation of Short-Term Assays.

DR. CONE: In a lot of the journals -- let's say essentially in all of the journals that we are using, they have been pre-screened for that. And this is something that we may have to do just because of the volume of the material. We are not doing it right now, not consciously.

PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Retrospective Cohort Mortality Study of Goldminers

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RETROSPECTIVE COHORT MORTALITY STUDY OF GOLDMINERS

I. Introduction

Most commercial forms of asbestos have been shown to have the potential for causing lung cancer, mesothelioma, and asbestosis. However, the pathogenicity of asbestos fibers appears to depend on the mineral type and specific size of the fibers. It is thought that long fibers, 5 microns in length, are the most pathogenic.

Exposure to non-commercial forms of asbestos are also of great concern. These exposures occur as a contaminant in mining operations and the fibers may be shorter than 5 microns in length. It is important to determine the health effects from the non-commercial forms of asbestos because of the possibility of such exposures in industry and among the population in general; and the results of such research are an important ingredient in setting standards for safe levels of exposure.

A large number of people were exposed to a non-commercial form of asbestos when tailings from a taconite mine were dumped into Lake Superior beginning in 1955. Subsequently, cummingtonite - Grunerite (CG) fibers, an amphibole fiber which is believed to be a type of amosite asbestos, was found in the drinking water in Duluth, Minnesota. At the same time it was known that CG was also present in the ore of the Homestake Goldmine. Because of the difficulty in conducting a good epidemiologic study (with a long enough latency period) of Duluth residents, it was decided to study the miners from Homestake which had been in operation for one hundred years.

Prior to the study under discussion here today, two other epidemiologic studies were conducted at Homestake. The first of these studies was conducted by NIOSH (Gilliam et al). (Slide 1) This was a mortality study which included 440 white males who had been examined by the U.S.P.H.S. during a silicosis survey and who had worked for at least five years at underground mining, and never mined elsewhere. These men were followed from April 1960 to December 31, 1973. Mortality rates from South Dakota were used as a standard in calculating expected deaths. An increased risk for respiratory cancer was observed, as well as for non-malignant respiratory disease.

The second study which was also a mortality study, was conducted by Dr. Corbett McDonald (Slide 2) and included all employees who worked for 21 years or more as of 1973. The workers were divided into five dust exposure classifications from very low to very high. For the study cohort overall, the risk for mortality due to respiratory cancer was not increased. There was a high risk for pneumoconiosis and respiratory T.B. For most causes of death, including respiratory cancer, the risk was slightly higher for those in the high dust exposure categories.

Because of certain inadequacies of both studies, a third study was proposed, and the preliminary results of this study will be discussed today. The epidemiologic study was carried out under contract by SRI.

The questions being addressed in this study are as follows. (Slide 3)

II. I.H. Survey

In order to relate the outcome of the epidemiologic study with specific occupational exposures, a detailed industrial hygiene study was conducted by NIOSH which I would like to briefly summarize. (Slide 4) The average length of the fibers are shorter than 5 microns, only 24% of CG fibers were >5 microns. At the time of the I.H. study the TWA exposure to fibers >5 microns was well below the standard. (Slide 5) These exposures were probably higher in the past, since the historical data on total dust shows a substantial decline after 1951, and it appears that all exposures are related to the level of dust exposure.

Other exposures in the mine including radon daughters and arsenic were below those levels thought to induce adverse health effects. Predictions of dust and fiber exposure for previous years have been made, however, the mortality analysis by specific exposure grouping has not been done at this time.

III. Methods

The epidemiologic study was designed as a retrospective cohort mortality analysis. The definition of the study cohort (Slide 6) was all Homestake workers who were employed full-time underground in the mine department for one year or more between January 1, 1940 and December 31, 1964. All of the mine records were screened to select the study cohort and company personnel records were used to supplement the data when necessary.

All miners who also had mining experience in uranium mines were excluded from the cohort. For each miner, the jobs held at the mine were coded into six categories, one of which included all work experience not considered a full-time underground job, such as work in the mill or in the office.

Once the cohort was selected and coded onto a computer file, an attempt was made to determine the vital status of each miner as of June 1, 1977. This effort was accomplished by searching records of the company, NIOSH records from the previous study, SSA, IRS, South Dakota Department of Welfare, South Dakota Department of Motor Vehicles, and the Post Office. For all those identified as being deceased, the death certificates were requested from the State Vital Statistics Office and the underlying cause of death was coded by a nosologist according to the ICDA Revision in effect at the time of death.

The mortality analysis was conducted using a person-years life table computer program developed by NIOSH. In the analysis, person-years (PY) at risk of dying were calculated for each miner, where PY's start accumulating after the miner qualified for the study cohort or in other words, after he worked one year in full-time underground mining jobs after January 1, 1940. The PY's accumulated until the study end date (June 1, 1977) or until the date of death, whichever occurred first. The PY's for all miners were then distributed into 5-year age groups and 5-year calendar-time periods and were multiplied by the corresponding U.S. white mortality rate to yield the number of deaths expected if the miners were dying at the same rate as the U.S. population. In addition, the life table analysis permits an examination of mortality by latency (time since first employment in a full-time underground job) and by duration of employment in full-time underground jobs. Observed deaths were compared with those expected and the difference was tested according to a poisson distribution.

IV. Results and Discussion

The total cohort consisted of 3144 miners. The results of vital status follow-up are given in Slide 7. Miners with an unknown vital status were considered alive as of the cutoff date. Of the 827 deaths, death certificates were not obtained for 94 miners. Slide 8 summarizes the observed and expected deaths for major causes of interest. The SMR is a measure of relative risk and is calculated by dividing the observed deaths by those expected and multiplying by 100. Any SMR above 100 indicates that the study population has an increased risk of mortality relative to the U.S. population. Note the statistically significant increased risk for T.B., respiratory disease (other than influenza, pneumonia and bronchitis) and accidents (other than transportation, poisoning, and falls). The risk for all cancers is close to that expected; the risk for heart disease and stroke is slightly lower than expected, which may be due to the healthy worker effect. The increased risk for all deaths is primarily due to the large excesses in T.B., respiratory disease, and accidents.

The mortality by specific cancer site was also examined. (Slide 9) Two sites had large SMR's -- cancer of the peritoneum and unspecified digestive organs, and cancer of the respiratory system other than the lung -- both of which were based on small numbers. Both of these sites are of interest because of the possibility of peritoneal and pleural mesotheliomas which are known to be caused by asbestos.

Hospital reports for these cases have been requested for additional information on actual cause of death. The risk for lung cancer mortality is very close to normal, but will be looked at in more detail later. There is an elevated risk for prostatic cancer and hematopoietic cancer.

Several causes of death were examined in more detail to determine whether or not their distribution by latency and duration of employment is consistent with an etiology associated with occupational exposure from underground mining at Homestake.

Tuberculosis

There was almost a four-fold excess in respiratory T.B. which included occupational lung diseases such as silico-tuberculosis. When examined by duration of employment (Slide 10) in full-time underground jobs, there is an obvious trend of increasing risk with increasing duration. There is also a positive trend showing an increasing risk related to increasing latency. (Slide 11) Both of these trends provide strong evidence that the increase in mortality from respiratory T.B. is associated with past employment underground at the goldmine. It should be noted that the high SMR for respiratory T.B. is also related to the date first employed underground. All but one of the observed deaths occurred among miners who were first employed prior to 1935.

Lung Cancer

Overall, the risk of mortality from lung cancer is only slightly increased (42 obs. vs. 40.7 exp.). However, because of its importance in the study, lung cancer mortality was looked at by examining the risk by duration underground after 15 years of latency had elapsed. (Slide 12) There is an increase in risk between 10 and 20 years of duration, however, the risk drops off after 20 years. This trend does not suggest that lung cancer mortality is associated with employment in underground jobs at the mine.

Leukemia and Aleukemia

Since the risk for leukemia and aleukemia was elevated it was also examined by duration of underground employment. (Slide 13) There was no restriction by latency period since it is thought that the latency period for leukemia may be very short. There is an increasing risk for this cause of death after 10 years of employment -- however, this is based on small numbers. It should be noted that diseases of the blood and blood forming organs also showed a two-fold risk.

Other Respiratory Diseases

There was almost a three-fold excess in respiratory diseases other than influenza, pneumonia, and bronchitis. Based on hospital reports, approximately two-thrids of these deaths are due to silicosis. No asbestosis cases were identified.

There is a positive trend of increasing risk with increasing lengths of employment underground (Slide 14), and most of the deaths and the increase in risk occurred after 30 years of latency. (Slide 15) As with respiratory T.B. these trends indicate that this cause of death is associated with employment underground and appears to be related to silica exposure.

Accidents

There is a high risk of mortality from accidents among the miners. When examined by duration of underground employment (Slide 16) there is a negative trend -- those with short-term employment have the highest risk. This relationship indicates that either new employees are given more dangerous jobs, or with experience, a miner learns how to avoid accidents.

V. Conclusions (Slide 17)

A verification of the file has been carried out to determine if all eligible miners have been included. A recent visit to the mine where all mine records were re-screened resulted in the selection of approximately 250 additional individuals. However, these individuals were randomly scattered throughout the files and varied in length of employment and dates first employed. This addition to the cohort should not substantially change the results.

Based on the current file we can conclude that underground miners have experienced high mortality for respiratory T.B. and non-malignant respiratory disease, both of which appear to be related to silica exposure. There is also a high risk of mortality from accidents.

Other diseases of concern include leukemia and aleukemia. According to these preliminary results, lung cancer mortality is not more than expected and there is no positive trend associated with duration of employment underground.

VI. Recommendations (Slide 18)

1. Add missing records and reanalyze.

2. Analyze specific mortality by intensity of exposure to dust and CG using the I.H. data collected.

3. Analyze by subcohorts according to date first employed to determine if changes in exposure have resulted in changes in mortality.

4. Continue follow-up, especially for those first employed after 1951 -- when dust levels were substantially lowered.

Slide 1

RESULTS OF THE GILLIAM ET AL. STUDY

Cause of Death	Observed Deaths	Expected Deaths	SMR
All Deaths	71	52.9	134
All Cancers	15	9.7	155
Respiratory Cancer	10	2.7	370**
Vascular Lesions of CNS	6	3.2	188
Heart Disease	25	25.2	99
Non-Malignant Respiratory	8	3.2	250*
Accidents	8	5.2	154

* Significant at $p < 0.05$
** Significant at $p < 0.01$

Slide 2

RESULTS OF THE MCDONALD STUDY

Cause of Death	Observed Deaths	Expected Deaths	SMR
All Deaths	631	549.7	115
All Cancers	93	90.5	103
Respiratory Cancer	17	16.5	103
Vascular Lesions of CNS	64	63.0	102
Heart Disease	264	232.5	114
Pneumoconiosis	37	--	--
Respiratory T.B.	39	3.6	1083*
Accidents	19	28.3	67

* Significant at $p < 0.01$

Slide 3

QUESTIONS BEING ADDRESSED IN HOMESTAKE STUDY

1. Is the cause specific mortality among underground miners different from expected mortality based on U.S. rates?

2. If excess cause specific mortality is found, can it be associated with a particular exposure?

3. In particular, is exposure to non-commercial asbestos fibers at the exposure levels experienced by Homestake miners, a significant factor in causing cancer?

Slide 4

CHARACTERIZATION OF THE ASBESTOS FIBERS

1. Cummingtonite - grunerite fibers:

   24% were > 5 micrometers in length
   Geometric mean diameter = 0.45 micrometers
   Geometric mean length = 3.3 micrometers

2. Tremolite - actinolite fibers:

   32% were > 5 micrometers in length
   Geometric mean diameter = 0.27 micrometers
   Geometric mean length = 4.1 micrometers

   Other fiber types include Hornblends and ambiguous or nonasbestos fibers

Slide 5

TIME WEIGHTED AVERAGE EXPOSURE TO FIBERS > 5 μm
AMONG HOMESTKAE MINERS

Job Category	Range (fibers/cm^3)	Geometric Mean
Miners	0.17 - 0.54	0.44
Underground Jobs Excluding Miners	0.05 - 2.50	0.24
Surface Work	0.12 - 5.34	1.16

Slide 6

DEFINITION OF THE STUDY COHORT

All Homestake workers who were employed full-time underground in the mine department for one or more years between January 1, 1940 and December 31, 1964.

Slide 7

Table 1

VITAL STATUS OF MINERS IN HOMESTAKE MORTALITY STUDY

Alive	2137 (68%)
Dead	827 (26%)
Unknown	180 (6%)
Total	3144

Slide 8

Table 2

MORTALITY OF MINERS IN THE HOMESTAKE STUDY
BY SPECIFIC CAUSE OF DEATH

Cause	Observed Deaths	Expected Deaths	SMR
Respiratory T.B.	36	9.5	379*
Other T.B.	3	0.5	575*
All Cancers	126	131.9	96
Diseases of Blood and Blood Forming Organs	3	1.6	183
Diseases of Nervous System	39	47.1	83
Heart Disease	265	317.7	83
"Other" Respiratory Disease	47	18.0	262*
"Other" Accidents	55	16.8	328*
Suicide	11	19.3	57
All Deaths	827	720.1	115*

* The difference between the observed and expected deaths is statistically significant at $p < 0.05$.

Slide 9

Table 3

MORTALITY OF MINERS IN THE HOMESTAKE STUDY BY SITE SPECIFIC CANCER

Cause	Observed Deaths	Expected Deaths	SMR
All Cancers	126	131.9	96
MN of Digestive Organs and Peritoneum	31	39.3	79
MN of Peritoneum & Unspec. Dig. Organs	2	0.6	333*
MN of Larynx	2	2.1	97
MN of Trachea, Bronchus and Lung	42	40.7	103
MN of Other Respiratory	3	0.5	633*
MN of Prostate	11	7.1	155
MN of Urinary Organs	2	7.2	28
MN of Other & Unspecified	13	16.5	79
MN of Hematopoetic Syst.	16	13.1	122
Leukemia & Aleukemia	9	5.6	161

* The difference between the observed and expected deaths is statistically significant at $p < 0.05$.

Slide 10

Table 7

MORTALITY OF MINERS IN THE HOMESTAKE STUDY FOR RESPIRATORY T.B. BY DURATION OF EMPLOYMENT UNDERGROUND

Duration In Years	Observed Deaths	Expected Deaths	SMR
1 - 5	0	2.9	--
5 - 10	2	2.3	99
10 - 15	5	2.0	279
15 - 20	10	1.2	939*
20 - 25	11	0.7	1671*
25 - 30	5	0.4	1362*
30+	3	0.2	1666*

* The difference between the observed and expected deaths is statistically significant at $p < 0.05$.

Slide 11

Table 8

MORTALITY OF MINERS IN THE HOMESTAKE STUDY FOR RESPIRATORY T.B. BY LATENCY

Latency in Years	Observed Deaths	Expected Deaths	SMR
< 5	0	0.8	--
5 - 10	0	1.2	--
10 - 15	0	1.5	--
15 - 20	3	1.6	183
20 - 25	4	1.5	271
25 - 30	6	1.1	536*
30+	23	1.8	1295*

* The difference between the observed and expected deaths is statistically significant at $p < 0.05$.

Slide 12

Table 9

MORTALITY OF MINERS IN THE HOMESTAKE STUDY FOR LUNG CANCER BY DURATION OF EMPLOYMENT UNDERGROUND FOR THOSE WITH 15 OR MORE YEARS OF LATENCY

Duration in Years	Observed Deaths	Expected Deaths	SMR
1 - 5	12	11.6	104
5 - 10	7	8.8	79
10 - 15	9	7.3	124
15 - 20	9	4.3	212
20 - 25	1	3.0	33
25 - 30	1	1.7	58
30+	1	1.3	77
Total	40	38.0	

Slide 13

Table 10

MORTALITY OF MINERS IN THE HOMESTAKE STUDY FOR LEUKEMIA AND ALEUKEMIA BY DURATION UNDERGROUND

Duration in Years	Observed Deaths	Expected Deaths	SMR
1 - 10	3	3.2	93
10 - 20	4	1.6	253
20+	2	0.8	255

Slide 14

Table 11

MORTALITY OF MINERS IN THE HOMESTAKE STUDY FOR "OTHER" RESPIRATORY DISEASES BY DURATION UNDERGROUND

Duration in Years	Observed Deaths	Expected Deaths	SMR
1 - 5	5	4.9	102
5 - 10	7	4.4	160
10 - 15	7	3.7	189
15 - 20	11	2.0	551*
20 - 25	5	1.5	342*
25 - 30	10	0.8	1202*
30+	2	0.7	286

* The difference between the observed and expected deaths is statistically significant at $p < 0.05$.

Slide 15

Table 12

MORTALITY OF MINERS IN THE HOMESTAKE STUDY FOR "OTHER" RESPIRATORY DISEASES BY LATENCY

Latency in Years	Observed Deaths	Expected Deaths	SMR*
1 - 5	0	0.1	--
5 - 10	0	0.3	--
10 - 15	1	0.6	175
15 - 20	0	1.1	--
20 - 25	2	1.8	110
25 - 30	9	2.4	376*
30+	35	11.7	300*

* The difference between the observed and expected deaths is statistically significant at $p < 0.05$.

Slide 16

Table 13

MORTALITY OF MINERS IN THE HOMESTAKE STUDY FOR ACCIDENTS BY DURATION UNDERGROUND

Duration in Years	Observed Deaths	Expected Deaths	SMR
1 - 5	29	8.1	359*
5 - 10	13	3.7	352*
10 - 15	8	2.5	321*
15 - 20	3	1.3	237
20+	2	1.3	157

* The difference between the observed and expected deaths is statistically significant at $p < 0.05$.

Slide 17

CONCLUSIONS

1. Missing records from file.
2. Based on current file the risk of mortality is high for:
   - o Respiratory T.B. (Including Silica T.B.)
   - o Non-malignant respiratory disease -- silicosis
   - o Accidents
3. Other diseases of concern
   - o Leukemia and Aleukemia
4. Lung cancer mortality is not more than expected in the cohort and there is no increase in risk with increase in duration.

Slide 18

RECOMMENDATIONS FOR FUTURE ACTIVITY ON THE HOMESTAKE STUDY

1. Add missing records to file and reanalyze.
2. Analyze the risk of cause specific mortality by intensity of exposure.
3. Do additional follow-up of the cohort first employed after 12/31/51 to determine if the high risk of non-malignant respiratory disease decreases or not.
4. Do analysis by subcohorts of date first employed.

DR. YODAIKEN: I think that tuberculosis is traditionally associated with gold mining, particularly with silica. So there must be other populations with which you could compare your results, outside of the United States. I wonder if you thought of doing that to see if there is a higher incidence at the Home State Mine as opposed to other goldmining communities, not necessarily in the States.

If you are considering follow-up studies on this group, one other thing that I should have thought you would include, is a study of nutrition because if ever there was a relationship between disease and nutrition, it is among tuberculotics. Have you anything in mind along those lines?

DR. BROWN: Well, a lot of these guys were employed 20, 30 years ago. I don't know how we could get that kind of information on the individuals since it is done historically from records.

All we did was go into the mine, identify the person by his personnel records, and there was never any contact made with the person. I don't know where we would get that information on an individual basis.

DR. YODAIKEN: This is not a problem now?

DR. BROWN: I don't know. I don't know if it is or not. That is why I would like to look at cohorts by when they first began employment, to look at a newer cohort to see if some of the respiratory problems have gone away or not.

DR. INFANTE: We are all sitting here and looking at your study and wondering what is going on in terms of cancer and lung cancer, and I couldn't help but be struck by the number of attributable or excess deaths from accidents. You know, from 827 deaths you have 55 from accidents. I mean, if that difference was due to cancer we would all be totally alarmed, but it is due to accidental deaths. To me it just seems from a significant risk standpoint, you know, it is tremendously significant. Did these deaths occur over a long period of time? Were they from a few cave ins? And is NIOSH planning to take any steps in terms of industrial safety in these mines? We all resign ourselves to the fact, well, miners just die from accidental deaths? Or what is the next step here? It doesn't seem like there is a whole lot of emphasis on the accidental deaths.

DR. BROWN: Well, they did not all occur in one great big cave in. There has never been a cave in at the mine. They occurred sporadically throughout the follow-up period and it is a real problem and something that I think they are trying to deal with. And it is something that I don't think is going away like--you can control the dust exposure and you can control some of the other things in a mine, but it is hard to control for the accidents. But that is typical of most mining populations I think. You are right, that is alarming.

DR. CARNOW: I was wondering about the tuberculosis. Tuberculosis is not a compensable disease; silicosis is. And in a lot of the mines, coal and gold mines, the diagnosis of tuberculosis as the lung disease from which miners were suffering was made for a long time. It has only been in recent years that silicotuberculosis has been introduced as a diagnosis. As a matter of fact, groups of physicians working with United Mine Workers only began making that diagnosis in the early 1950's. So the fact that these people were diagnosed as tuberculosis is not surprising, particularly if this were ten or 15 years ago.

PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Support of a Workshop for the Cancer and Heart and Lung Disease Task Force

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SUPPORT OF A WORKSHOP FOR

THE CANCER AND HEART AND LUNG DISEASE TASK FORCE

Dr. Morris I. Kelsey (NCI) and
Ms. Peggy Y. Young (Geomet Technologies, Inc.)

Good afternoon, ladies and gentlemen. I would like to summarize for you today the results of a workshop entitled, "Exposure to Environmental Agents, their Metabolism and Mechanisms of Toxicity, Research Needs." This Workshop was funded via the NCI/EPA Collaborative Agreement and was held in late January of this year in Rockville, Maryland.

PURPOSE OF THE WORKSHOP/BACKGROUND

The purpose of this workshop was to draft research needs which would address the problems of Exposure, Metabolism and Mechanisms of Toxicity associated with environmental cancer and heart and lung disease. This effort was conducted by the Exposure and Metabolic Mechanisms Project Group which is part of the Task Force on Environmental Cancer and Heart and Lung Disease. This Task Force was established by Congress in the Clean Air Act Amendments of 1977 and is chaired by the Environmental Protection Agency with support from Geomet Technologies, Inc. Other members of the Task Force include representatives from NCI, FDA, NHLBI, NCHS, NIEHS, CDC and NIOSH. It should be appreciated that the Task Force is required to report its progress to the Congress annually and that is why the Workshop was convened: To rapidly respond to Congress by preparing research needs (recommendations) that should be studied by various agency directors for possible implementation.

In order to design a Workshop that would actually draft research recommendations, the following steps were implemented:

1. Members of the Project Group, of which I am privileged to Chair, selected a team of experts in various subject areas of interest and concern to the Task Force and in most areas the Chairpersons were Project Group members (I'll return to that issue later). The composition of the panels included representatives from government, academia and industry.

2. These scientists submitted short "Position Papers" in advance of the Workshop for distribution to the respective participants in each session of this Workshop, and the participants are listed in the front of this document. These papers were used for reference and discussion purposes only.

3. Small subgroups were then assembled at the Workshop to draft research recommendations with the accompanying statements of rationale. These recommendations were derived from a consensus of the subgroup panel.

4. All of the recommendations were discussed in two Plenary Sessions, and a consensus of the invited participants was obtained in order to prepare a "Rough Draft" of the Workshop document.

COMMENTS ABOUT WORKSHOP

It should be noted that the Workshop contained only one session of formal talks that were presented by prominent Health Scientists, in specific areas of expertise including:

- Dr. Robert Gordon - Special Assistant to the Director of NIH
- Dr. Jean French - CDC and a Project Group member
- Dr. Manning Feinleib - NHLBI and Working Group member
- Dr. John Higginson - Director of IARC
- Dr. Richard Dowd - Assistant Administrator for R&D, EPA at that time
- Hon. James Martin - Scientist and member, U.S. House of Representatives

The following three days of the Workshop consisted of closed subgroups sessions chaired by the following individuals:

1. Exposure - Epidemiology

   Dr. Tom Mason - NCI, Project Group member
   Dr. Paul Leaverton - NHLBI, ex-Working Group member, (Co-Chairperson)

2. Exposure - Quantification

   Dr. Herman Kraybill - NCI, Working Group and Project Group member
   Dr. Lance Wallace - EPA, Project Group member

3. Metabolism

   Dr. Jim Gillette - NHLBI, Project Group member
   Dr. Elizabeth Weisburger - NCI, Project Group member

4. Mechanisms of Toxicity/ Carcinogenicity

Since the Project Group did not have this in-house expertise, we were fortunate in recruiting Dr. Larry Loeb from the University of Washington as Chairman and Dr. James Trosko from Michigan State University.

Preparation of the final draft document was as follows:

- February 1981 - Project Group Reviews and Revision of Draft
- March 4, 1981 - Working Group Review and Revision
  - Delineation of Recommendations
    (1) Policy: to Congress
    (2) Scientific: To Task Force Agencies
- April 15, 1981- Plenary Group Review, Revision and Approval

Following the Plenary Group meeting, members of the Project Group and Task Force Working Group made minor modifications to the report. Two of the three Task Force Recommendations to Congress in their Fourth Annual Report were derived from our Workshop effort and included the following:

RECOMMENDATIONS 1. A high priority should be given to the implementation and continued operation of the National Death Index being developed under the auspices of the National Center for Health Statistics.

RECOMMENDATIONS 2. Legal impediments to both Federal and non-Federal epidemiology research should be studied further.

The subject areas where these scientific recommendations were developed and are shown below:

1. Exposure - Epidemiology
2. Exposure - Quantification
3. Monitoring Individual Human Exposures
4. Metabolism
5. In Vivo Toxicity
6. Mechanisms of Toxicity/Carcinogenicity

I will comment briefly about the recommendations in each of these areas.

1. Exposure - Epidemiology: 8 Recommendations

   Some important aspect of these recommendations include:

   - Problems in gaining access to Federal data systems which contain valuable information vital to epidemiologic research
   - Emphasis on epidemiology of human reproduction
   - Efforts to quantify relationship between exposure and response.

2. Exposure-Quantification (multi-media aspects): 10 Recommendations

   - Multidisciplinary approach to environmentally-induced disease
   - Use of biologic sentinels of the environment including domestic animals, fish, etc.
   - Assessment of influence of geochemical environment on disease
   - Sharing and integration of environmental data bases
   - Quantification of exposure from multi-media.

3. Methods of Monitoring Individual Exposures to Environmental Pollutants: 9 Recommendations

   - Stresses importance of methods development in assessing exposure (personal vs. fixed station monitoring)
   - Development of quality assurance program for government sponsored collection of environmental data
   - Measurement of total exposure, combined exposure, baseline monitoring for total body burden assessment, and coordination with epidemiology studies
   - Environmental monitoring of chemical emergencies.

4. **Metabolism**: 17 Recommendations

- Research is needed to determine ways in which genetic traits and environmental factors modify toxicity of environmental chemicals
- Importance of metabolism, pharmacokinetic parameters in response to various doses of test chemicals to understand mechanisms of toxic events
- Information is needed regarding metabolic pathways and kinetics of drug metabolizing enzymes to compare responses in animals and humans
- Emphasis on pharmacogenetic differences in metabolism are needed
- Attention to development of mathematical models of carcinogenesis for extrapolation of observations from animals to humans
- Long-range support for quantum chemical predictions of interactions between chemicals and receptor sites.

5. In Vivo Toxicity: 17 Recommendations

- More emphasis in teratology research including detection methods for teratogens and development of data bases on comparative metabolism and pharmacokinetics to facilitate interspecies extrapolation
- Development of methods to monitor exposed populations for genetic effects
- More emphasis should be given to respiratory tract disease including physiological and morphological studies; study sites of major impact by toxic agents within respiratory systems and mechanisms of pathology associated with these diseases
- Exploration of toxicological interactions based on knowledge of metabolism and mechanisms of action of individual toxicants
- Effects of passive smoking on health and interaction with other toxicants.

6. Mechanisms of Toxicity and Carcinogenicity: 11 Recommendations

- Need studies of not only initiation, but on promotion of carcinogenesis and replication of undamaged and damaged genetic material
- Studies designed to minimize the somatic mutation rate via mutagen detection and elimination
- Develop methods to quantify somatic mutation rates in animals being tested with environmental chemicals in order to detect early endpoints of toxicity vs. tumorigenesis -- eventually extend methods for testing humans
- Develop procedures to measure accumulations of DNA-adducts and types of DNA damage in tissues of animals exposed to known toxicants and eventually extend to humans.

Thank you.

PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Mortality Study of Chemical Workers in the Kanawha River Valley Region of West Virginia - A Progress Report

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NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRAM ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES
September 10, 1981

MORTALITY STUDY OF CHEMICAL WORKERS IN THE KANAWHA RIVER VALLEY REGION OF WEST VIRGINIA - A PROGRESS REPORT

Terry L. Leet[1], Susan G. Austin[2], Richard J. Waxweiler[1],
and Robert A. Rinsky[1]

Introduction

In August 1979, the National Institute for Occupational Safety and Health (NIOSH) and the Union Carbide Corporation announced their decision to conduct and jointly sponsor a study to evaluate the mortality experience of all Union Carbide employees at three chemical plants in the Kanawna River Valley region of West Virginia. The decision to conduct a historical cohort mortality study of all Union Carbide Kanawha Valley employees was based partly on the results of a proportionate mortality study sponsored by Union Carbide and two other hypotheses involving occupational exposure to polycyclic aromatic hydrocarbons produced by coal hydrogenation and occupational exposure to ethylene oxide.

The Union Carbide-sponsored proportionate mortality study was based on 819 deaths that occurred between 1965 and 1978 among active and retired male employees of one Union Carbide Kanawha Valley plant (Marsh, 1979). The study revealed a statistically significant excess in cancer mortality and, more specifically, a two- to three-fold excess in multiple myeloma and cancer of the kidney and central nervous system (Table 1). However, due to the methodological limitations of the porportionate mortality study, i.e., incomplete ascertainment of deaths among all employees who worked in the plant and the use of proportions rather than rates in determining expected numbers of death for the cohort, the study results were used to generate hypotheses of potential exposure and disease for the entire Union Carbide Kanawha Valley population.

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[1] National Institute for Occupational Safety and Health, Cincinnati, OH.
[2] Union Carbide Corporation, New York City, NY.

Both NIOSH and Union Carbide were interested in evaluating the potential health risks associated with exposure to chemical compounds produced by coal hydrogenation. The exposure was mainly from oil products with very high boiling temperatures, e.g., polycyclic aromatic hydrocarbons, aromatic amines, toxic metals, and organo-metallic compounds (Koppeneal and Manahan, 1976; Sexton et al., 1960). A number of studies have indicated an association between many of these chemicals and occupational cancers of the respiratory system, urinary system, and skin (Carter and Roe, 1975; Freudenthal et al., 1975). In 1960, an Union Carbide plant physician, reported a high incidence of skin cancer among 359 coal hydrogenation workers who were examined regularly over a five-year period (Sexton et al., 1960). In 1979, a subgroup of 50 Union Carbide coal hydrogenation employees, who developed cutaneous precancerous lesions and/or skin cancer, were examined in a mortality study. No adverse risks were seen, possibly due to the very small sample size (Palmer, 1979). Both NIOSH and Union Carbide were interested in expanding this study by identifying all Union Carbide employees associated with the coal hydrogenation pilot plant in West Virginia, but were faced with the problem of screening the employment records for approximately 44,000 active and inactive employees. Incidentally, the same screening problem existed in 1974 when NIOSH was attempting to identify a cohort of workers exposed to vinyl chloride at the Union Carbide Kanawha Valley plants.

In 1976, NIOSH had initiated a feasibility study with the intent of locating a cohort of workers occupationally exposed to ethylene oxide. Ethylene oxide was of interest to NIOSH for three reasons. One reason was the toxicological evidence of mutagenicity in at least thirteen biological species following exposure to ethylene oxide, which was later summarized in the 1977 NIOSH Special Occupational Review on ethylene oxide (Glaser, 1977). The second reason was the potential exposure among chemical workers and other occupational groups due to the high production and worldwide use of ethylene oxide as an intermediate in the manufacture of several industrial products. The third reason was the lack of epidemiologic studies in the literature at that time, concerning the potential risks of workers exposed to ethylene oxide. Coincidentally, Union Carbide had already initiated a mortality study of ethylene oxide workers. When Union Carbide learned of NIOSH's interest, Union Carbide allowed NIOSH to conduct the study. However, during the execution of the study, NIOSH realized the screening did not include all workers exposed to ethylene oxide, since all ethylene oxide departments were not initially identified. Therefore, to avoid selection bias in the ethylene oxide cohort study, to determine the chronic health effects of all Union Carbide employees associated with the coal hydrogenation plant, and to investigate the reported excess cancer mortality in the Union Carbide Kanawha Valley population, Union Carbide and NIOSH agreed to study all workers who were ever employed at the three Union Carbide Kanawha Valley plants between January 1, 1940 and December 31, 1978.

Purpose

This study will evaluate the mortality experience of all Union Carbide Kanawha Valley employees by determining 1) whether there is an excess in mortality from multiple myeloma or cancer of the kidney, brain, or liver and biliary passages in any of the three plant-specific cohorts, 2) whether there is an excess in mortality from leukemia and aleukemia in the ethylene oxide exposure cohort, and 3) whether there is an excess in mortality from cancer of the respiratory system, urogenital system, or skin in the coal hydrogenation cohort, when compared to the mortality experience of a similar segment of the general population.

Description of the Plants

The study will focus on the populations of three Union Carbide plants, specifically South Charleston, Institute, and Technical Center. The South Charleston plant, the first facility owned by Union Carbide in the Kanawha Valley, began operation in November 1925. As a chemicals and plastics production facility, it has been involved over the years in the manufacture of a wide variety of substances including ethylene oxide, polyethylene, vinyl resins, and polyols.

The Institute plant was originally built by the United States Government as part of the Rubber Reserve Corporation for the production of styrene and butadiene, which were basic raw materials needed for the production of synthetic rubber. Production began early in 1943 and the facility continued as a United States Government operation until 1947 when it was sold to Union Carbide. Under Union Carbide operation, the plant was used as a larger production facility for materials developed at the South Charleston plant, e.g. acetone, isopropanol, butanol, and acetaldehyde. From 1951 to 1956, it maintained a coal hydrogenation pilot plant for the production of numerous aromatic and aliphatic chemicals. In more recent years, the Institute plant has grown to include the manufacture of agricultural chemicals, e.g., SEVIN[R] insecticide, and a wide range of ethylene oxide and propylene oxide derivatives.

The Union Carbide Technical Center was built in 1949 as a facility to house one of its chemicals and plastic research departments. In 1958, the facility was expanded to include a development and engineering department. The Technical Center has been responsible for the research and development of ethylene oxide and its derivatives.

Methods

The total cohort, which is composed of all three plant populations, includes approximately 44,000 Union Carbide employees. This number contains 10,000 active and 34,000 inactive employees. All employees were identified through personnel and payroll records. Demographic and work history data have been coded by Union Carbide and are being computerized by NIOSH.

A departmental dictionary correlating departmental codes and chemicals used or produced in each plant was prepared by Union Carbide to facilitate the construction of the ethylene oxide and the coal hydrogenation cohorts. The departmental dictionary contains the chemicals used or produced in each production unit by year and the appropriate alpha and/or numeric departmental codes for the production unit.

Ascertainment of cohort vital status, collection of death certificates for an estimated 6,800 deceased employees, and data analysis will be conducted using standard procedures, which have been described in other reports given today. However, additional analyses may include a series of nested case-control studies for specific diseases of interest in any of the previously described cohorts. The purpose of nested case-control studies will be to determine whether the disease excess is associated with the work environment or with other personal or environmental factors.

It is anticipated that the cohort analyses will be completed by mid-1983.

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Table 1

Proportionate Mortality Ratios and Proportionate Cancer Mortality Ratios
for Selected Causes Among Male Chemical Workers Hired Prior to 1955+

Cause of Death	Obs	PMR United States	 West Virginia	 Kanawha County	PCMR United States	 West Virginia	 Kanawha County
All Malignant Neoplasms	223	126.6**	146.1**	131.9**	-	-	-
Digestive System	54	113.4	144.9**	134.1*	90.0	100.3	102.8
Liver and Biliary Passages	8	270.6**	251.8**	212.9*	220.1*	183.8	172.1
Kidney	12	273.8*	377.2**	385.6**	217.3**	255.6**	291.3**
Brain and Other CNS	9	203.1*	280.5**	244.8**	165.7	206.5*	228.2*
All Lymphopoietic Cancer	27	173.2**	218.4**	195.0**	138.7	153.9*	147.6*
Multiple Myeloma	9	NA	448.9**	425.0**	NA	320.8**	322.0**

*p<.05 **p<.01 NA = Data not available to compute PMR and PCMR
+Reference: Marsh, 1979

PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Mortality and Industrial Hygiene Study of Workers Employed in the Leather Tanning and Finishing Industry

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Mortality and Industrial Hygiene Study of Workers Employed in the Leather Tanning and Finishing Industry

Introduction

The following paper is intended to: (1) briefly review the literature concerning the health effects caused from working in the leather industry; (2) discuss the processes and exposures found in the industry; and (3) provide an update of the current study being conducted.

Several case control studies have indicated that individuals employed in the leather industry may have an increased risk of developing bladder cancer.

Cole et al.$^{(1)}$ compared the occupational histories of 356 Eastern Massachusetts males newly diagnosed with carcinoma of the lower urinary tract (malignancy of the renal pelvis, ureter, bladder, or urethera) to a random sample of adult males from the same geographic area (Slide 1). Twenty-one of the study group reported that their usual occupation was employment in the leather or leather products industry, whereas only 12.1 would have been expected to have their employment in that industry, an increase of 70%. When analysis was made according to ever having worked in the leather or leather products industry the relative risk for cancer of the lower urinary tract elevated to 2.25, a 125% increase over that expected based on matched controls (Slide 2).

In another study, Decoufle[2], through a set of data maintained by Roswell Park Memorial Institute (RPMI), a major cancer research center in Buffalo, New York, retrospectively examined the lifetime occupational work histories of 6434 males and 7515 females who developed cancer between 1956 and 1965. The risk of developing cancer from a given occupation was compared against persons whose lifetime occupational history consisted entirely of clerical jobs. The highest relative risk for bladder cancer among all occupational groups studied, was demostrated in the category "operatives in the leather industry". Out of all the bladder cancer cases diagnosed among males at the Roswell Park Memorial Insitute, between 1956 and 1965, 11 had been employed in the leather industry during their working lifetime: a six fold increase over what would have been expected (Slide 3). When analysis was conducted by just those persons working 5 years or longer in the leather industry, the relative risk for bladder cancer increased to 12.94. Excess mortality was also observed for cancer of the buccal cavity and pharynx and cancer of the larynx.

In another case control study, Decoufle[3] observed that among male residents of Fulton County, New York the mortality from bladder cancer was 21% greater than that of New York State and 53% higher than that of the United States. Decoufle wanted to ascertain whether this excess may have been due to the large concentration of leather tanneries within the Fulton County area. All death certificates from bladder cancer for the period 1958 - 1976 were collected and 2 matched controls for each case were chosen whose underlying cause of death was anything other than cancer. The results revealed that forty-one percent of the males worked in the leather tanneries compared to 28% of the controls, a relative risk of 1.66.

In view of the previous studies which show an unusual cancer experience for workers in the leather industry, the National Institute for Occupational Safety and Health (NIOSH) began a historical review of the industry including the processes used and the potential chemical exposures that exist.

The leather industry as we know it today, began in the 19th century (Slide 4). The industry grew steadily until 1965 but because of imported finished leather products, the industry has somewhat declined. Currently, the tanning industry employs a total of almost 19,000 persons in 250 plants, and processes a total of 17 million hides per year. A located map points out where the plants presently exist within the United States (Slide 5).

As depicted on the preceeding slides, the leather industry is very labor intensive, with almost 19,000 employees in 250 plants; and, although some automation has been introduced, the workers are still required to handle wet hides throughout the entire tanning process. There a variety of potentially hazardous substances workers may encounter within the tanning industry (Slide 6). These include chromium compounds, nitrosamines, dyes, and solvents among others. Some of these agents will be examined as a review the steps involved in the tanning process are described.

Process Description

The tanning process begins at the hide house where the delivered salted hides (calf, cattle, or sheep) are split into two sides, sorted, trimmed, and graded (Slide 7). From here, the sides are moved to the beam house where they are soaked with a wetting solution to remove excess salt, dirt and blood and to restore moisture that has been lost.

After soaking, the sides are fleshed to remove excess fat and muscle and then dehaired. It is in the dehairing process that the chemical dimethylamine sulfate (DMAS) is used. DMAS has been found to be a precursor for N-nitrosodimethylamine (NDMA), a known and regulated carcinogen. NDMA has been found in air samples taken from various leather tanneries.

After dehairing, the hides are relatively clean and ready for tanning. The sides are placed into tanning drums with a chromium compound to remove the natural oils of the leather and replace them with a preservative.

Hexavalent chromium componds, which are often used on the lighter skins, have been linked with cancer of the respiratory system.

Following the tanning process, the sides are spit into uniform thickness and shipped to the color and fatliquor department, where the sides are put into drums and dyed into various colors. A multiplicity of coal tar dyes (aniline dyes) have been used, including azo dyes, which have been shown to be carcinogenic in animals.

The hides are then either toggled onto metal frames or pasted onto metal sheets and processed through a drying oven to remove excess moisture and to be stretched.

The hides are then transferred to the buffing and conditioning department where the leather is tempered for firmness and sanded for smoothness. The application of the final finish is another possible source of exposure to carcinogens. Pigments made from azo-dyes which are used on leather in the finishing process, have been found to be carcinogenic in animals. Solvents such as benzene and toluene has also been found in use with the finish mixer spayer machine.

Since there are various areas in the leather industry where potentially hazardous substances exist, NIOSH felt it advisable to more clearly assess the industry.

Methodology

A retrospective cohort study design was chosen to analyze whether employees in the leather industry may have an increased health risk over that of the general U.S. population.

Two leather plants in the same general area of the Midwest were selected. These two plants were preferred because of (Slide 8):

(1) the number of years the plants and processes of interest had been in existence. Both plants had started operations about 1900 allowing time for latency as well as length of employment to be analyzed.

(2) the number of employees both historically and presently who had worked at the plants. One of the plants chosen had over 2,800 employees who had worked at the facility over the years, while the other plant had close to 7,000. This will allow for subgrouping of the cohorts by department and/or operation.

(3) the completeness and adequacy of the record systems. Both plants selected maintain record systems dating back to the 1940's, which are complete for demographic and work history information. Stratification based upon age, sex, date first employed, date terminated, and department can be analyzed.

(4) the knowledge that one company used dimethylamine sulfate whereas the other one did not. One of the interests in the study will be to try and determine whether the use of DMAS presents a potential health risk and this will allow for that type of analysis.

The cohort being studied to ascertain the health effects of workers employed in the leather tanning industry consists, then, of about 9500 males who were employed between January 1, 1940 and December 31, 1977.

Personnel records of all prior and current employees were microfilmed and a computerized file consisting of all demographic and work history data was generated.

Vital status ascertainment is being traced for each number of the cohort from the time of termination of employment till December 31, 1977. At the present time, vital status of 72% of the cohort has been determined (Slide 9).

A modified life table analysis technique will be used to obtain race, sex, and calendar year specific person-years-at-risk (PYAR). PYAR will be calculated from the first date employed till the study end date of December 31, 1977 or death, whichever occurs first for each study member. These PYAR will be multiplied by the cause-specific death rates of the United States to obtain the number of expected deaths. The expected deaths will be compared to the observed number of deaths and a two-tailed Poisson distribution will be employed to evaluate statistically significant differences.

Data analysis will include variables of latency, length of employment, and job category, among others.

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OBSERVED AND EXPECTED NUMBERS OF MALE CASES, RELATIVE RISKS, AND ASSOCIATED CONFIDENCE LIMITS, ACCORDING TO CATEGORY OF "USUAL OCCUPATION[1]"

	MALE CASES			
OCCUPATION CATEGORY	OBS.	EXP.**	REL. RISK	95% C.L.
01. DYESTUFFS	0	1.8	-	0.00-2.43
02. RUBBER	19	11.6	1.65	0.82-3.35
03. LEATHER	21	12.1	1.70	0.86-3.34
04. PRINTING	5	7.7	0.75	0.29-1.94
05. PAINT	11	8.7	1.31	0.57-2.97
06. PETROLEUM PRODUCTS	34	34.4	1.05	0.65-1.71
07. OTHER ORGANIC CHEMICALS	3	2.0	1.62	0.32-8.25
08. OTHER CHEMICALS	6	6.9	0.92	0.34-2.46

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[1] TAKEN FROM COLE, ET. AL.

**CONTROLLED FOR AGE

OBSERVED AND EXPECTED NUMBERS OF MALE CASES, RELATIVE RISKS, AND ASSOCIATED CONFIDENCE LIMITS, ACCORDING TO "EVER EMPLOYED" IN OCCUPATIONAL CATEGORY[1]

	MALE CASES				
OCCUPATION CATEGORY	OBS.	EXP.**	EXP.	REL.RISK	95% C.L.
01. DYESTUFFS	6	4.0	3.5	2.33	0.66-8.24
02. RUBBER	46	33.7	32.5	1.63	1.04-2.56
03. LEATHER	65	32.7	34.3	2.25	1.46-3.46
04. PRINTING	14	13.9	15.1	1.30	0.68-2.49
05. PAINT	23	21.8	24.6	1.19	0.69-2.05
06. PETROLEUM PRODUCTS	79	75.3	79.0	1.18	0.82-1.69
07. OTHER ORGANIC CHEMICALS	13	10.9	9.5	1.44	0.74-2.80
08. OTHER CHEMICALS	17	19.7	19.6	0.99	0.54-1.81

** CONTROLLED FOR AGE

+ CONTROLLED FOR AGE AND CIGARETTE SMOKING

[1] COLE, ET. AL.

CANCER EXPERIENCE OF MALES EMPLOYED IN THE LEATHER INDUSTRY - ROSWELL PARK MEMORIAL INSTITUTE, 1956 - 1965+

	EVER EMPLOYED		EMPLOYED AT LEAST 5 YEARS	
	CASES	RELATIVE RISK	CASES	RELATIVE RISK
BLADDER CANCER	11	6.77**	8	12.94**
BUCCAL CAVITY AND PHARYNX	18	3.378**	12	3.772**
LARYNX	7	3.633*	6	6.896**

+ TAKEN FROM DECOUFLE ET. AL. (3)

* SIGNIFICANTLY DIFFERENT FROM UNITY AT THE .02 LEVEL.

** SIGNIFICANTLY DIFFERENT FROM UNITY AT THE .01 LEVEL.

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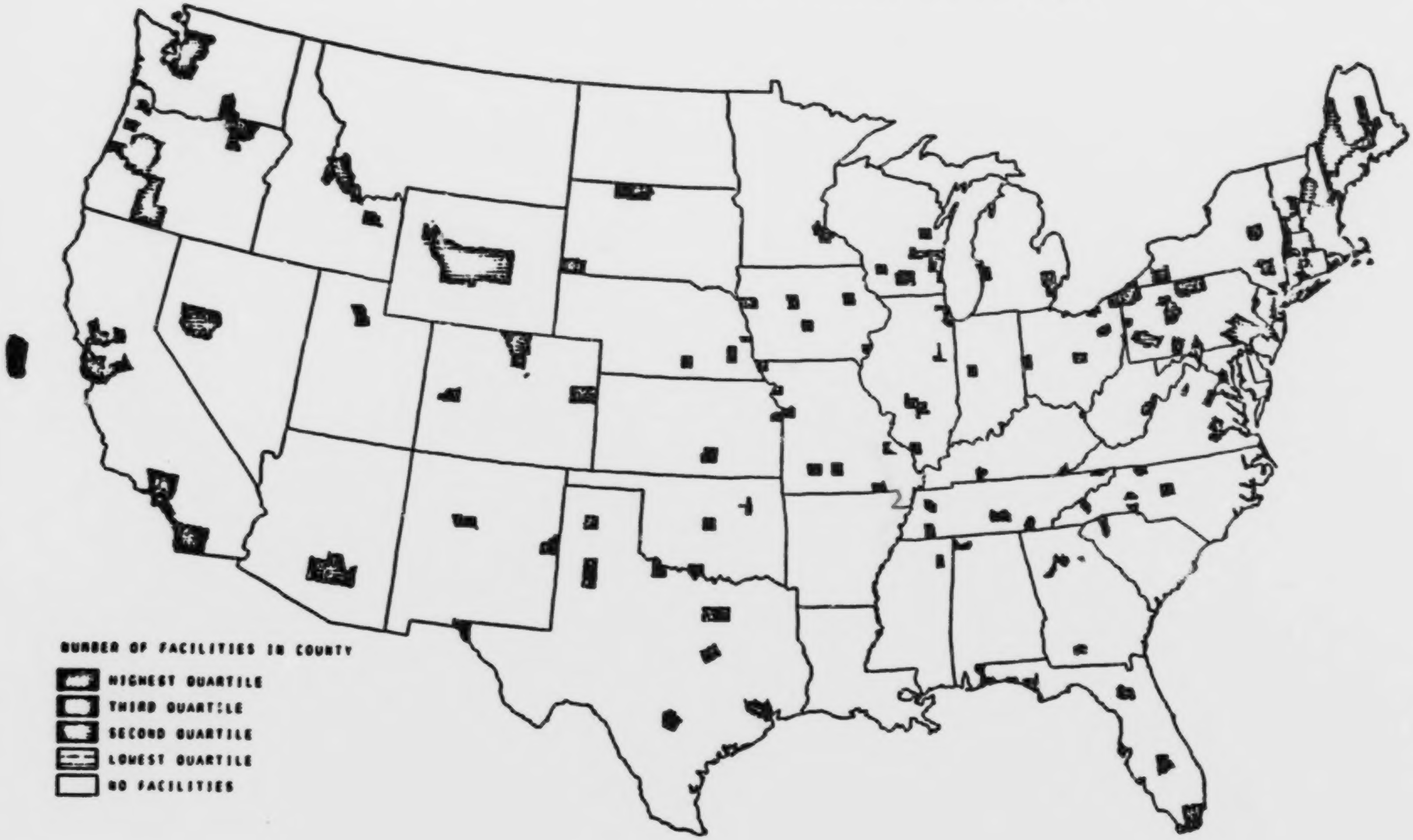
TANNERIES IN THE UNITED STATES[1]

	TOTAL # OF PLANTS	TOTAL # OF EMPLOYEES	TOTAL # HIDES PROCESSED
1965	*	32,700	32,700,000
1980	250	18,903	17,600,000

[1] FROM DR. ROBERT LOLLAR, TECHNICAL DIRECTOR,
NATIONAL TANNING COUNCIL

*NOT KNOWN

NIOSH INDUSTRY LOCATOR MAP
HAZARD SURVEILLANCE SECTION 1980
S.I.C. 3111 LEATHER TANNING AND FINISHING


NUMBER OF FACILITIES IN COUNTY
HIGHEST QUARTILE
THIRD QUARTILE
SECOND QUARTILE
LOWEST QUARTILE
NO FACILITIES

TANNING INDUSTRY

SUBSTANCES TESTED IN IN-DEPTH INDUSTRIAL HYGIENE SURVEY

- HEXAVALENT CHROMIUM
- TRIVALENT CHROMIUM
- NITROSAMINES
- DYES-BENZIDINE DERIVED
- SOLVENTS
  - n-BUTYL ACETATE
  - BUTYL CELLUSOLVE
  - METHYL ETHYL KETONE
  - METHYL ISOBUTYL KETONE
  - TOLUENE
  - XYLENE
  - ACETONE

TANNING INDUSTRY

PROCESS FLOW OUTLINE

1. RECEIVING SALTED HIDES
2. TRIMMING AND SORTING
3. SOAKING
4. FLESHING
5. UNHAIRING
6. TANNING
7. SLITTING
8. COLOR AND FATLIQUORING
9. DRYING
10. CONDITIONING
11. BUFFING
12. MEASURING AND GRADING
13. FINISHING
14. SHIPPING

TANNING INDUSTRY

PLANTS CHOSEN FOR STUDY

	BEGAN OPERATION	NO. OF PRODUCTION EMPLOYEES CURRENT/PREVIOUS	COMPLETENESS OF RECORD SYSTEM MAINTAINED	DIMETHYLAMINE SULFATE (DMAS) USAGE
PLANT A	1908	277/2821	SINCE 1941	NO
PLANT B	1858	155/6602	SINCE 1933	YES
RESULT	ANALYZE BY LATENCY AND LENGTH OF EMPLOYMENT	SUBGROUP BY DEPARTMENT AND/OR OPERATION	STRATIFY BY AGE, SEX, DATE EMPLOYED, DATE TERMINATED	DETERMINE EFFECT OF DMAS

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VITAL STATUS OF COHORT OF LEATHER WORKERS

			TOTAL NO.	%
TRACED ALIVE			6516	69.1
TRACED DECEASED			1177	12.5
DEATH CERTIFICATES OBTAINED	498	42.3		
DEATH CERTIFICATES OUTSTANDING	679	57.7		
LOST TO FOLLOW-UP			1730	18.4
TOTAL			9423	100.0

MS. BRAVER: I am Elisa Braver, OSHA. My understanding from speaking with John Fajen is that occupational exposure to hexavalent chromium no longer existed in tanneries because the two-bath process had been phased out. Would you comment on this?

DR. STERN: Yes, John Fajen is the industrial hygienist on this project. Hexavalent chromium is not used as much as it was in the past but it is still used in some tanneries. A couple of tanneries that John went to did use hexavalent chromium and they have since closed down. In fact, there are quite a few tanneries that have closed down just in the last couple of years. There are quite a few other substances in the leather tanning industry, 200 or 300 other substances, in fact, that have been tested mainly by OSHA. We are not going to be able to look at all of them but we will be able to look at some of the ones that John sampled for, which include the ones that I have mentioned.

PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Mortality and Industrial Hygiene Study of Workers Exposed to Toluene

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Mortality and Industrial Hygiene Study of Workers Exposed to Toluene

Introduction

The following paper is intended to (1) breifly review the background information of toluene; (2) discuss the literature concerning the health effects; (3) provide reasons for pursuing a study; and (4) update the progress of the current study (Slide 1).

Toluene, an aromatic hydrocarbon, also known as toluol or methylbenzene, is a clear colorless noncorrosive solvent with a sweet odor similar to that of benzene (Slide 2).

Approximately 96% of all toluene that is produced comes from petroleum and petrochemical processes with about 4% from coal-tar oil. In 1978, the annual production rate for toluene exceeded 5 billion pounds.

The current Occupational Safety and Health's (OSHA) standard for toluene exposure is 200 parts per million (ppm) with a ceiling level of 300 ppm. The National Institute for Occupational Safety and Health's (NIOSH) recommended standard for toluene is 100 ppm with a ceiling level of 200 ppm.

The uses of toluene are many with over 50% of toluene being produced converted to making benzene. The remaining uses of toluene are for producing other solvents, explosives and gasoline (Slide 3).

Highly purified toluene is presently used in many commercial and industrial products and contains less than 0.01% benzene as a contaminant (Slide 4). Industrial grade toluene, however, may contain significant quantities of benzene, as high as 25%.

Studies of Toluene Exposure

Various animal and human studies have been conducted to evaluate the health effects from exposure to toluene (Slide 5). The studies of toluene exposure prior to 1940, however, were complicated by the frequent contamination of toluene with benzene. Benzene, although structurally and chemically similar to that of toluene - toluene has one more methyl group ($-CH_3-$)-has been previously associated with blood and bone marrow disorders including anemia and leukemia, myeloid metaplasia, and chromosomal aberrations (Slide 6).

Animal Studies

Animal experiments after 1940 indicate that toluene is more irritating and, in lower concentrations, has a somewhat stronger narcotic action than benzene but that its effect on the blood and blood forming organs is considerably less severe. In general, daily 4- to 8-hour exposures of up to 1000 ppm of toluene produced little or no significant effect in different animal species.

Human Studies-

Effects on the Hematopoietic System

The effects of toluene on the hematopoietic system of humans are of concern since toluene is a methylated benzene and benzene is a known leukemogenic substance. However, various studies conducted to evaluate the effects of toluene on the hematopoietic system have generally shown no myelotoxic effects although some controversy still exists.

Effects on the Central Nervous System

Acute exposures to toluene have been shown to depress the central nervous system resulting in headache, nausea, muscular weakness, fatigue, mental confusion and, at extremely high exposures, coma and death. In an experimental study, Von Oettingen[7] exposed three healthy individuals to various levels of toluene exposure 8-hours a day. He found that at concentrations of 200 ppm definite impairment of coordination and reaction time resulted which he felt would render affected persons dangerous to themselves and the safety of others. Von Oettingen's work has led to the current Occupational Safety and Health's standard for toluene of 200 ppm.

Other Effects

Renal disease resulting from exposure to toluene has also been reported by several investigators. O'Brien[6] in 1971 described a case of a 19 year old male who had been sniffing glue containing 80% toluene for 3 years and who had developed serious, but reversible injury to his kidneys. Another researcher[3] reported a similar finding.

Other factors that may determine the health effects experienced from exposure to toluene are the uptake and elimination of the inhaled solvent. Although men seem to show a larger uptake of toluene than women, men also excrete the substance at a much faster rate. In addition, women only eliminate about one half as much toluene as men. This is thought to be due to the increased body fat in women. Therefore, women seem to maintain a higher level of toluene than men when exposed to similar levels.

Rationale

There are various reasons for continuing to investigate the health effects from toluene exposure (Slide 7):

(1) Toluene, as stated previously, is chemically similar to benzene, a recognized carcinogen. However, it wasn't until the 1940's when industry recognized the health risks of benzene and began substituting other solvents in its place. Although toluene is not known to have the same leukemogenic effects as benzene, it is recommended that this be further confirmed in human epidemiologic studies.

(2) Previous animal and human studies on the effects of toluene exposure have been relatively few and often times have been complicated by the frequent contamination of toluene with benzene.

(3) The Interagency Testing Committee of the Toxic Substances Control Act, representing 8 governmental health agencies, was established in 1977 to identify those substances which require additional research to assess their impact upon human health. From the Committee's list of 3650 substances identified as potential health risks, 10 were recommended for further study. The criteria used in their evaluation included production volume, number of persons exposed, both occupationally and non-occupationally, environmental release, and similarity to other substances already known to present a health risk. Toluene was one of the 10 substances recommended for furtner study.

(4) Up to the present time, there has been little information on the chronic effects in humans from exposure to low levels of toluene over an extended period of time. With the long term use of toluene (since the 1940's) and a large population at risk, an epidemiologic study would be feasible.

Study Design

A retrospective cohort mortality study was the design chosen by the National Institute for Occupational Safety and Health (NIOSH) to ascertain the long term health risks of former workers exposed to toluene. Several industries using toluene were considered in an attempt to isolate a "pure" exposure. Considered were adhesive manufacturing, shoe manufacturing, leather manufacturing, bookbinding, furniture industry, cabinet makers and the rubber industry among others. The shoe manufacturing industry was selected as a toluene adhesive user for several reasons (Slide 8):

(1) No industry was identified as only using toluene.
(2) The exposures to other agents appeared minor.
(3) The industry had not been well studied in terms of occupational safety and health problems.
(4) The industry was well suited for an epidemiologic and detailed industrial hygiene study.

Selection of plants within the shoe industry was based upon: (1) type of adhesive used (solvent based and/or water based); (2) no previous benzene use; (3) number of years of use of the solvent-based adhesives; (4) number of workers exposed, both past and current; and (5) availability and completeness of records.

Several health related problems had been previously reported in the literature for the shoe and footwear industry (Slide 9).

(1) Nasal cancer (adenocarcinoma of ethmoid sinuses) has been associated with leather dust exposure in the shoe industry in Great Britian.

(2) Gynecological disorders among female workers in the footwear industry has been associated with posture, standing and sitting, age, and length of employment.

(3) Noise and vibration problems from machines and automated processes have produced hearing loss.

(4) Various chemical expousres have produced a variety of health related problems in the shoe industry.

   (a) N-hexane exposure has produced peripheral neuropathy

   (b) Acetylene tetrachloride has been associated with acute yellow level atrophy.

   (c) A variety of solvents such as toluene, MEK, acetone, and xylene have caused central nervous system disorders.

Process Description

The procedure for turning raw materials into a marketable pair of shoes involves 8 principle steps (Slide 10). Initially, the raw materials consisting of leather and man-made fabrics, as well as soles and heels, etc. are received, separated, graded, and sorted in the receiving department.

The leather is then transferred to the cutting department where various sized dies are used to conform the leather into the different shoe styles required. Skilled workers are employed in the cutting process to keep waste to a minimum. No dyes or finishes are used in this department.

The cut material then is sent goes to the fitting department where the upper parts of the shoe are constructed. Leather pieces are either sewn or glued together and bows, tassels and other ornaments are added. Adhesives are used in several of these operations.

In the sole department, the shoe soles are roughed with a wire brush and cement is applied. Primary operations include roughing, edging, grinding, and cementing. The operation may involve running the sole through an adhesive roller and stacking the soles on wire racks to dry. Exposures received here would be from solvents and wood dust.

In the lasting department, the upper part of the shoe is stretched over a "dummy foot" called a last and tacked into place.

Soles, once dried, are brought to the bottoming department where they are cemented onto the upper part of the shoe. In addition, the cementing of steel shanks, outsoles, and heels are performed in the bottoming department.

In the making department, the assembled shoe is removed from the last and finishing touches and cleaners are applied. Various solvents are used in this department.

Finally, the shoes are packaged and sent out to the customers.

Environmental toluene samples using a draeger pump and detector tubes were taken in all the shoe plants surveyed. An example of some of the findings is shown in Slide 11.

Methodology

The cohort being studied to ascertain the health effects of working in the footwear industry consists of almost 10,000 white females who were employed sometime between January 1, 1940 and December 31, 1977 in two shoe manufacturing plants within the midwest.

Personnel records of all prior and current employees were microfilmed and a computerized file consisting of all demographic and work history data was generated.

Vital status ascertainment is being traced for each member of the cohort from the time of termination of employment until December 31, 1977. At the present time, vital status of 74% of the cohort has been determined (Slide 12).

A modified life table will be used to obtain race, sex, and calendar year specific person-years-at-risk (PYAR). PYAR will be calculated from the first date employed until the date of death or study end date of December 31, 1977 for each member of the study cohort. These PYAR will be multiplied by the cause-specific death rates of the United States to obtaint the number of expected deaths. The expected deaths will be compared with the observed number of deaths and a two tailed Poisson distribution will be employed to evaluate statistically significant differences.

Data analysis will include variables of latency, length of employment, age at beginning date of employment, and job category, among others.

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TOLUENE STUDY

PRESENTATION IS INTENDED TO:

BRIEFLY REVIEW THE BACKGROUND INFO. OF TOLUENE.
DISCUSS LITERATURE CONCERNING HEALTH EFFECTS.
PROVIDE REASONS FOR PURSUING A STUDY.
UPDATE PROGRESS OF NIOSH STUDY.

TOLUENE STUDY

GENERAL INFORMATION

SYNONYMS: METHYLBENZENE, TOLUOL

CHEMICAL FORMULA: $C_6H_5CH_3$

DESCRIPTION: COLORLESS LIQUID WITH A
BENZENE-LIKE ODOR

PRODUCED FROM: 96% PETROLEUM
4% COAL-TAR OIL

PRODUCTION RATE: 5 BILLION LBS./YEAR

CURRENT OSHA STANDARD: 200 PPM. T.W.A.;
300 PPM. CEILING

NIOSH RECOMMENDED STANDARD: 100 PPM. T.W.A.;
200 PPM. CEILING

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Page

TOLUENE STUDY

QUANTITATIVE DISTRIBUTION OF USES

	PERCENT
BENZENE	51
SOLVENTS	10
EXPLOSIVES	9
ISOCYANATES	5
PHENAL	1
GASOLINE POOL AND MISC.	24
	100

TOLUENE STUDY

TOLUENE IS PRESENT IN:

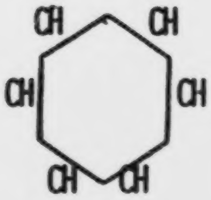
- ADHESIVES - MODEL AND CHINA CEMENT, CONSTRUCTION ADHESIVE
- PAINT AND VARNISH REMOVERS
- STAIN REMOVERS AND DRY CLEANERS
- NAIL POLISHES
- INKS - PERMANENT MARKERS
- FUEL SYSTEM ANTIFREEZE
- PAINTS AND PAINT THINNER
- ASPHALT REMOVER
- METAL CLEANER
- ANTHELMINTIC - VETERINARY

Studies of the Effects of Toluene Exposure

Animal Studies	Authors
Central Nervous System	Lehmann[5]
Human Studies	
Hematopoietic System ?	Capellini,[2] Browining,[1] Wilson[8]
Central Nervous System	Von Oettingen[7]
Renal Disease	O'Brien[6], Ehrenreick[3]
Liver Dysfunction	Greenburg[4]

CHEMICAL STRUCTURE OF BENZENE AND TOLUENE

BENZENE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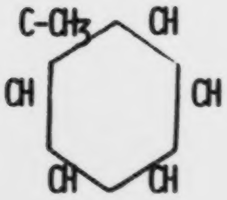
TOLUENE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TOLUENE STUDY

REASONS FOR INVESTIGATING THE HEALTH EFFECTS

1. CHEMICALLY SIMILAR TO BENZENE, A KNOWN CARCINOGEN
2. PREVIOUS STUDIES HAVE BEEN COMPLICATED BY TOLUENE BEING CONTAMINATED WITH BENZENE.
3. INTERAGENCY TESTING COMMITTEE OF TSCA RECOMMENDED TOLUENE BE STUDIED FURTHER
   - A. PRODUCTION VOLUME : 5 BILLION LBS./YR
   - B. NUMBER OF PERSONS EXPOSED : 5 MILLION
   - C. ENVIRONMENTAL RELEASE : 1 BILLION LBS./YR
   - D. SIMILARITY TO KNOWN CARCINOGEN : BENZENE
4. NO INFORMATION ON CHRONIC EFFECTS IN HUMANS FROM LOW-LEVEL EXPOSURE TO TOLUENE OVER A LONG PERIOD OF TIME

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TOLUENE STUDY

REASONS FOR SELECTION OF SHOE MANUFACTURING INDUSTRY

NO INDUSTRY IDENTIFIED AS ONLY USING TOLUENE.

EXPOSURE TO OTHER AGENTS APPEARED MINOR.

INDUSTRY NOT WELL STUDIED IN TERMS OF HEALTH PROBLEMS.

INDUSTRY WELL SUITED FOR EPIDEMIOLOGIC STUDY.

Toluene Study

Health Problems Reported in the Literature for the Shoe & Footwear Industry

Leather Dust: Nasal Cancer

Ergonomic Factors: Standing & sitting postures have caused gynecological disorders

Noise & Vibration: Machines & Automated processes have produced vibration disease and hearing loss

Chemical Exposures:
T O C P, N-hexane-Polyneuritis
Isocyanates (TDI)-Lung Disorder
Acetylene Tetrachloride-Acute yellow
Liver atrophy
Adhesives & Cleaning Solvents-CNS

TOLUENE STUDY

SHOE MANUFACTURE
-PROCESS FLOW-

RECEIVING

CUTTING

FITTING

SOLE

LASTING

BOTTOM

MAKING

PACKING

SHIPPING

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TOLUENE STUDY

AIR SAMPLING RESULTS

(DRAEGER PUMP AND DETECTOR TUBES) 11-28-79

MEASUREMENT NUMBER	SAMPLE LOCATION	TOLUENE DRAEGER TUBE READING (PPM)
1	CEMENT INSOLE OPERATIONS - INSOLE DEPARTMENT	70 - 75
2	CEMENT HEELS - MAKING DEPARTMENT	50
3	CEMENT OUTSOLES - INSOLE DEPARTMENT	70 - 80
4	INSOLE CEMENTING - (SOCK LINE TO INSOLE) - INSOLE DEPARTMENT	100
5	CEMENT OUTSOLES - INSOLE DEPARTMENT	250
6	BETWEEN CEMENT INSOLE (MANUAL AND MACHINE CEMENTING) - INSOLE DEPARTMENT	70 - 75
7	CEMENT HEELS - MAKING DEPARTMENT	50
8	AUTOMATIC SHANKING MACHINE - WELT DEPARTMENT	40
9	HAND CEMENT UPPERS - FITTING DEPARTMENT	25

VITAL STATUS OF COHORT IN THE SHOE MANUFACTURING INDUSTRY

		TOTAL	
		No.	%
TRACED ALIVE		5736	58.3
TRACED DECEASED		1516	15.4
DEATH CERTIFICATES OBTAINED	1176		
DEATH CERTIFICATES OUTSTANDING	340		
LOST TO FOLLOW-UP		2586	26.3
TOTAL		9838	100.0

385 **BEST DOCUMENT AVAILABLE**

DR. WEISBURGER: There is one important difference between benzene and toluene, that is, toluene has that nice side chain which can be oxidized to a carbocylic acid and then conjugated with glycene and so forth. So, you know, to compare them just on the basis of structure isn't exactly cricket.

DR. STERN: Right, they metabolize differently.

SPEAKER: Have you thought of looking at reproductive effects?

DR. STERN: There is some possibility of looking at some of the medical effects. I think it is possibly going to depend on what results we find in this study. There are other studies that are being conducted though on reproductive effects of various solvents.

PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Compilation of 1972 Mortality Data

John Patterson

National Center for Health Statistics
3700 East-West Highway
Hyattsville, MD

COMPILATION OF 1972 MORTALITY DATA

My remarks are more of an announcement than a report. As many of you know, back when the National Center for Health Statistics was processing the death certificates for the 1972 national mortality file we ran into very serious budgetary and personnel problems. At the time, we processed only a 50 percent sample of the death certificates for 1972.

This presented a number of problems for Tom Mason at the National Cancer Institute and several other people at NCI and the EPA, particularly with their county mapping studies and also with the follow-up studies that were based on these studies.

A year or so ago they negotiated a reimbursement agreement with the National Cancer for Health Statistics to have us go back and process the remaining 50 percent of the 1972 file.

We have completed the coding and keying of the remaining 50 percent of the records. We are now in the process of editing and verifying the results. We expect to be able to deliver a computer tape on the second 50 percent of the mortality file to the National Cancer Institute in October. This file will be available to others who would be interested in using the remaining 50 percent of the 1972 mortality file.

If any of you would be interested in obtaining copies of this tape, you can get in touch with me at the Division of Vital Statistics at the National Center for Health Statistics.

PROCEEDINGS OF THE
SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

Thursday Afternoon, September 10

Plenary Session

Session Chairperson:
Dr. Richard Marland
Environmental Protection Agency

DR. MARLAND: I am Richard Marland and we will convene a combination of plenary session, plus, concurrent discussion of the day's papers.

We will try and arrange this in a manner to provide you with a good overview, with those ideas that are perceived as being most useful for future directions for research, and at the same time, provide you with some views of the persons here who have chaired these various sessions.

Let me speak extremely briefly because I want this whole thing to last no more than 30 minutes, if possible, let me speak only in terms of how epidemiology is viewed by a regulatory agency. I think I can speak in terms of the OSHA needs. I can certainly speak in terms of EPA's needs.

When it comes to the enforcement of some kind of an environmental standard or an industrial standard for the protection of life, the highest quality data is alleged to be that which comes from epidemiolgical studies. Therefore, we constantly seek the highest quality data to support the standards which we are required to enforce.

It has been my perception today, as it has been for many years, that the problems of conducting epidemiological studies that are without flaws have not yet been solved. I hope that we can be concerned with this because more and more we need unflawed epidemiology.

To open up this session I would like to call on the afternoon concurrent discussants to accomplish their summary of the afternoon, together with pointing toward future directions. And I will call first on Ralph Yodaiken from NIOSH in terms of the epidemiology part from Group A.

DR. YODAIKEN: I am actually not going to be able to summarize the papers for two reasons: First of all, I have not had the time and, secondly, I am not a particularly good note taker. So I am going to make some comments that are not all necessarily pertinent to the afternoon's presentations but cover both aspects that I am interested in and facts that came out in the morning's papers.

One is the obvious necessity of correlating some of the studies that are being carried out, with the nutritional status of the people who are collaborating in the studies, namely the workers and the controls.

I was particularly struck by Dr. Mason's attempt to relate Vitamin A and D to carcinogenicity. I have a criticism of that study, and I am sorry he is not here to talk about it. If we are going to look at the nutritional status, I think it is important to recognize that there is a latent period when it comes to cancer. To look at nutritional studies in terms of Vitamin A and D levels in the blood today and try to relate those to cancer incidence, may be fallacious.

I think it would be appropriate in studies of this kind to try and back track and determine nutritional status ten years or 15 years ago where there may have been some deprivation or some starvation period which perhaps would be more significant than current blood levels.

I was also very impressed with the study that was presented, and I think we all agree, with Dr. Peters. Dr. Peters' study on the epidemiology of populations previously exposed to hexachlorobenzene was both beautifully presented and was an excellent example of how epidemiology should be done.

But one of the suggested remedies put forward was that perhaps it might be a good idea to stop breast feeding because of the presence of hexachlorobenzene in breast milk. Now, although this is not NIOSH's particular field, I think it would be a mistake for us as scientists to ignore the social implications of making suggestions of that kind.

In a case like that, the suggested remedy may be more drastic than the disease itself. And, in any case, this question has arisen in other studies of the effects of pesticides on reproduction. How do we know that the breast milk is the factor and how do we exclude the possibility that the fetus has not already acquired a body burden that produces the disease in the first year of life, or early in life?

So some of the studies that I would like to see initiated in Turkey wherever similar pesticide investigations are being carried out in the United States, would be to determine body burdens in stillborn fetuses and in products of conception where there are abortions.

Finally, I would be erring in my own particular dedication if I didn't draw attention to the fact that in the Washington Post yesterday there appeared an announcement that the Reagan administration was considering doing away to a large extent with warning foreign countries who buy toxic products from the United States. They are going to modify the policy that regulates chemicals, such as PCB's, chlorofluorocarbons, banned pesticides, DDT, and so forth; these are no longer going to be exported with an appropriate warning. I think that this flies in the face of both our obligations as scientists and also the fact that the United States is reducing itself in status from a leader in the field of environmental control to that of a follower.

The statement that appeared in the Post stated that the United States' exports are at a competitive disadvantage since other people don't warn Third World countries or underdeveloped countries of the hazards of the products which they buy, and therefore, we should do the same. It is our obligation as a scientific community to express wherever we can, the dismay that we feel about that kind of policy. I think it is entirely inappropriate for the United States to also export those consequences of hazardous substances, that Dr. Peters demonstrated are possible, to other countries.

DR. MARLAND: Thank you. In terms of keeping the presentations consistent, Tom Mason had the epidemiology portion of the first part this morning and has left me his notes. With your permission, I will provide you with the six recommendations which Dr. Mason has prepared for this conference. They are quite simple. I think some of them are quite good and provocative in terms of our thinking.

One: "The collaborative programs should be utilized to pursue population-based studies in areas of the United States with cancer registries to identify high risk groups."

Number two: "The collaborative programs are reasonable resources to fund a multi-disciplinary team to evaluate and assess emergent health problems."

This is somewhat -- not off the wall exactly -- but it is an unprecedented use of a resource of this nature in my opinion -- a ready team, ready to lunge at incidents and accidents and provide the benefit of several kinds of skills, including monitoring, as well as epidemiological analyses. Quite an interesting recommendation.

Here is number three: "Studies funded by the collaborative programs should not focus solely on cancer, but rather on a broad base of diseases." On the basis of today's papers I think that recommendation is being fulfilled and, of course, there are many of us who welcome and appreciate that.

Recommendation number four: "Projects should be funded to develop methodologies such as developing an appropriate set of comparison rates for spontaneous abortions among working women."

This one, combined with another one, makes me want to make a comment as well. But number five first: "Projects should be funded to develop dose-response data." That one is fairly straight forward.

Number six: "More rapid laboratory methods should be developed to measure health effects of environmental exposures." This could be combined with number four. I would have consolidated them. From these points made by Dr. Mason, and my own observation, it appears to me that the methods used for environmental epidemiology should not and need not be substantially the same as those that we use for industrial or occupational, and certainly not similar to those which we are using for traditional, communicable disease type of epidemiology. I am afraid that looking at it as an administrator and not as a participant in the studies, there is a lack of common agreement among environmental epidemiologists as to what does constitute rules of good, acceptable epidemiologic studies for environmental measurements, particularly at the low doses and the chronic exposures that we have to deal with.

I would, therefore, suggest if I could amend Tom's comment it would be to address the need for more highly defined criteria for assessing good environmental epidemiology at chronic exposure low dose rates.

Now let's move to the two persons who have been responsible for the Group B sessions and I will again start with the afternoon participant. John?

DR. COOPER: I find it difficult to summarize the discussion. I have two reactions to what was presented in this group of papers which probably address future needs of our program. Perhaps by commenting on them I can stimulate some discussion.

The first point that struck me very strongly was the importance and utility of the data base generation which was described in New Jersey. If, indeed, such data base generation efforts can be stimulated in other states in some sort of a uniform format, there is a tremendous potential for epidemiologic rapid response to emerging questions. I think it is a dramatic development within the program area, and I would think one of our future directions might be to attempt to stimulate more work in this area.

The other thing that impressed me -- I have no answer -- but in looking at the studies that were presented to us today, I noticed the remarkably large loss to follow-up in all of the cohort studies. I really think that we have to find some way to resolve this problem because when one looks at a study with a 26 percent loss to follow-up it is very unlikely that worst case analysis will allow us to draw any conclusions unless the relative risks were so dramatic, as have been seen with vinyl chloride.

So I am very disturbed with that and it seems to me one of our future directions has got to be to attempt to find ways to reduce this level of loss to follow-up. Those are my comments, I believe, on the paper I heard in the second session. Perhaps we will have some more discussion on them.

DR. MARLAND: Thank you. The fourth presentor speaking for Group B this morning, of course, is Tom Cameron from the Cancer Institute. Tom?

DR. CAMERON: Well, I am more fortunate than John was. I had a discussion. My problem was, I was so interested in the discussion I didn't make good notes. However, I think I have crystalized our half-hour discussion. I remember I initiated it by sticking my neck out and suggesting that some of the major epidemiologic studies that we have been exposed to might better have been preceded by some lab work. I did get some reaction.

From there we went into a general discussion and I have about four points on that situation. We did decide that a lot more work should be devoted to metabolic pathways. The compound of discussion was styrene.

Then there was some discussion of identifying biological markers and using them during the course of study to identify cohorts and possibly new cohorts.

We spent quite a bit of time discussing the needed development of short-term tests, whole animal tests, as well as others. I know we have the dominant lethal and the strain A mouse assays, but they all have their limitations and perhaps we haven't followed them long enough or intensively enough. I know that there is an undercurrent of uneasiness with those tests.

Perhaps we can make a concerted effort to aid in developing further whole animal tests. Incidentally, along that line, John Couch is with us now and he will be tomorrow presenting his efforts with the fish, which I find personally very, very interesting. We have been supporting that activity for three years and John now tells me that he is ready to give us the word.

With the other short-term in vitro tests, as we all know, there are certain weaknesses in detecting metals and dusts and other classes of compounds.

We sort of summed up this whole situation of the effort or collaboration between epidemiology and lab tests by suggesting that perhaps we should put them in parallel. I think that is a good idea. I don't think any extensive epidemiological study should be initiated without a fair background in lab activities.

We recognized the fact that we had in our audience a few people from industry and we welcomed them and we very quickly suggested that we work for more ways to encourage industry cooperation.

One point was made that processess are changing very rapidly in industry and it is only industry that can keep us apprised and current.

After we broke up I discussed one point with our industrial representatives and we had hoped to get to it in the discussion this afternoon and that was, "the hazards of collaborating with the U.S. Government."

To put it in football terms, for those of you who do follow football, I think what they had in mind was something about outlawing again the crack-back block. Is that what you had in mind? I hope to get to that tomorrow. I think it is very, very important. And if we can increase the cooperation we are receiving from industry now I think we are all going to profit.

Very quickly then, someone suggested that we take a look at the industrial hygiene practices. The thing that struck several of us this morning was the statement about washing the arms with acetone. I just wonder how many other practices like that are out there that we haven't detected.

The last point that we discussed was really sort of a little nudge at the Cancer Institute and I have to admit that I am probably one of the major offenders; that is, our tendency to focus so sharply on the cancer end point that we really eliminate everything else.

I will say that we are comfortable with mutagenicity but we do to date have been very uncomfortable with teratogenicity, as an example. Our discussion ended really with people suggesting, or urging strongly, that we expand the toxicologic end points in our studies, both in the laboratory and in epidemiology. I think that is about what I got out of it.

DR. MARLAND: Thank you, Dr. Cameron. I want to comment favorably and thank the three of you for what I find to be excellent summaries and useful recommendations.

I have a couple more chores that I would like to perform, one, Ken Bridbord and Joe Fraumeni have been laboring faithfully as your general chairmen and I am going to give them a minute or two at least to cogitate on whatever wrap-up remarks they would like to make. In the meantime, are there comments from the floor that are appropriate to the summaries prepared by the four group chairmen?

DR. KRAYBILL: I think Tom touched on it, but one thing that we did emphasize this moring in that session was the need for a system whereby the technical reports from bioassay are screened. I think that movement is underfoot. There is one chap that is looking over the bioassay reports now to exploit the findings; from those studies to, in turn, go to the epidemiologists and say, here, we've got something good. I think you ought to look at it.

That is to say, ever since I have been at NCI, I have had the feeling the experimentalists work over in their world. I have never seen an ideal situation where there is enough interfacing. I think the ideal situation would be to have the experimentalists talk to the epidemiologists and tell them about some of the findings that are coming out of the laboratory with the experimental animals. So I think we need to do more of this.

Then mention was made of using clinical parameters and, Dr. Cameron I think, touched on that, the bio-chemical markers so the epidemiologists can use this tool to sophisticate their studies.

DR. MARLAND: Thank you.

DR. GAFFEY: I am William Gaffey from Monsanto. One of the things that struck me in the presentations that were given today, particularly the cohort mortality studies, was that many of them are unfinished and always will be in the sense that the populations are small. Again and again we come to a report in progress in which it is clear that when the study is finished, unless there are rather spectacular findings, we will find ourselves saying we need a bigger population, or we need a longer period of observation.

It seems to me that the only solution to this kind of problem is to push the kind of operation that was mentioned briefly, that industry-wide studies are possible. It is possible to get larger cohorts if one can induce the industries concerned to contribute data.

The second problem allied to this is that we probably will find ourselves in the future going back to the same populations, following them longer. Again and again study recommendations say in effect, yes, we've done this but we recommend that we come back in ten years and do it again.

This means that more and more often in the future we are going to be doing cohort mortality studies in which 90 percent of the population has died. This is going to create real methodological problems. There is no point in going into them in great detail, except, for example, the business of competitive risk which is now a little thing that we give lip service to but which will become a great big problem when we go back to a cohort 40 years from now and find that 90 percent of them have died.

DR. MARLAND: Thank you. I sense a consensus arising from the group which I welcome and think is a great idea in terms of a closer relationship between industry's participation in these studies and government's paying attention to industry's participation. I think this is a very healthy sign personally.

Dr. Fraumeni, have you some summary words as chairman of this morning's session?

DR. FRAUMENI: I have about three points to make. One is that some of our summary speakers on the platform have been apologetic that we focus too much on cancer and that we should pay attention to other end points. I would like to counter that by saying that the emphasis of this program should be on environmental and occupational cancer studies. Needless to say, we should keep our eyes open to other health effects, including other chronic diseases, mutagenicity, teratogenicity, and so forth. But we still have plenty of problems to settle in the field of cancer, and I think we should keep in mind that this is the primary focus of the collaborative program.

The second point is that many of the projects that have been presented seem rather limited to single agency involvement. I think that as the program matures, we should take advantage of the strengths and the expertise of individuals in different agencies, not only NIOSH and EPA, but also agencies like NCHS and CDC, outside of NIOSH. Particularly as we get into multi-disciplinary investigations that require expertise in industrial hygiene, environmental health, laboratory investigations and epidemiology, it will become essential to combine the talents of investigators in different institutes and agencies.

The third point I would like to make is that whenever possible we should continue to utilize national data resources, and that we should combine our efforts to protect them and develop their application for epidemiologic studies. I am thinking of resources like the Social Security Administration, the Bureau of the Census, the Internal Revenue Service and the National Center for Health Statistics. Right down the line we have plenty of national data systems that can be valuable in developing, generating and evaluating etiological hypotheses. The National Death Index is now finally off the ground, but for its survival it will need to be supported and nourished by the different agencies.

DR. MARLAND: Thank you. I particularly appreciate your putting in good perspective the relationship between cancer as a major thrust of our activities in addition to the other health effects.

Dr. Bridbord will provide us with his recommendations.

DR. BRIDBORD: Thank you. There are a number of points I would like to make. I know that time is getting late. I think the decade of the 1980's is a very important decade for environmental epidemiology in general and for occupational epidemiology in particular. One reason I say that is, if one is somewhat familiar with chemical production in this country, one recognizes the fact that there have been major, major increases for many chemicals, particularly since about 1960. Because of the latency aspects, at least in terms of cancer, one may first begin to see effects through epidemiology in studies that begin to look at this point in 1980 and later.

I think in this regard one has to pay very, very close attention to cancer trends, to cancer trends for specific sites and specific age groups. In this regard, because of the latency situations, I think it is unlikely to find much evidence of occupational or environmental cancer in populations under age 45.

I think, though I firmly agree with the point made by Dr. Gaffey that we are going to have to go back and update all the cohorts and be careful about methodology in doing that, there will still be a need to more completely ascertain what happens to cohorts.

Many of the studies presented at the meeting, although preliminary reports, still, apart from the ascertainment and vital status issue, included at best 25 percent of the people, in the deceased category, and we still have to wonder what is going to happen to the other 75 percent.

I think one possible clue to occupational etiology may lie in a closer look in the differences in cancer rates for non-hormone dependent tumors, particularly between men and women, recognizing the fact that at least up till now the tendency has been for men to be employed more traditionally in jobs involving toxic exposures. I think that probably will be the trend in the future although employment patterns have changed in the recent past somewhat.

I think we also need to spend more time looking at what happens to minority workers in this regard, at least in certain industries it may be the minority workers who have the highest degree of exposure.

I think we also have to pay very, very close attention to which control groups we are using in our studies and to recognize that with the proliferation of chemical exposure and with the understanding that occupations may be more important contributors to cancer than previously recognized, the general population becomes a less satisfactory control group. We thus end up doing studies much akin to the following, looking at lung cancer rates in smokers and comparing them to the lung cancer rates in the general population, also including many smokers. While we would still pick up the fact that lung cancer is related to smoking. We may miss important implications in terms of the quantitative impact of that risk.

I think it is very, very important in both occupational and environmental studies to gain more accurate exposure information. We need to pay more close attention to including occupational histories in routine medical care and clinical histories. Here perhaps our colleagues at the Cancer Institute can help to encourage their own cancer centers to more routinely consider this factor.

We may be missing something by not looking at children of workers. I am particularly struck by recent data that John Peters and colleagues have generated from California about the possible relationship of occupation to cancer in children -- occupation of parents and cancer in children.

And finally, we need to keep in the back of our mind that the disease we see is very likely to be the result of interactions, interactions of environmental exposures, interactions of personal factors as well, including smoking and dietary habits. And we need to be thinking of ways that our epidemiologic, as well as our laboratory techniques, can pick these up. Thank you.

DR. MARLAND: Thank you, Ken. Are there comments from the participants in the room regarding either the summary comments of the chairmen or any other worthwhile comments for the good of all? Yes, Dr. Kraybill?

DR. KRAYBILL: No technical comment, just a logistical comment. I know that Dr. Adamson will be here tomorrow, and he had given us the admonition that he would like to have some input, some resource material. I think what was discussed right up here at the table was excellent. All I am saying is, if you have your remarks written down on paper, please don't throw them away. Save them because the front desk out there has offered to get them typed up. Having them typed up they will serve two purposes: They might be good for the people tomorrow in concurrent sessions and, particularly, for the chairman of the plenary session tomorrow afternoon that is Dr. Adamson, because there are some very good recommendations here. So if you will please hand your remarks in at the registration desk, we will get them typed then tomorrow morning.

DR. MARLAND: Thank you. Yes, Dr. Morris?

DR. MORRIS: Just a reminder, this is a bit of housekeeping, we have arranged for Dr. Arthur Sober, who was scheduled in the afternoon of tomorrow's session, to begin tomorrow at 8:45. He has received very short notice to do this so I hope we have a good turn-out at 8:45. He has problems in scheduling.

DR. MARLAND: Thank you. That is a good reminder. 8:45 a.m. is our beginning tomorrow. Let me extend my personal thanks to the persons here who have served as chairmen and have lent their talents and time to an important piece of work. Also, my thanks to the excellent speakers. I was personally very pleased with the level of competence and quality of the papers. And I look forward to tomorrow, 8:45. Thank you and good night.

PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

Friday Morning, September 11

Methodology, Experimental, and Models Session

Session Chairperson:
Dr. Ralph Yodaiken
National Institute for Occupational Safety and Health

PROCEEDINGS

DR YODAIKEN: Good morning early birds. For those of you who managed to make this early morning presentation, I am Ralph Yodaiken from NIOSH and I am your chairman for this morning. Our first paper will be presented by Dr. Sober from Massachusetts General Hospital. The subject is Dosimetry Studies in Selected Locations where Epidemiology Studies are Planned. The project officer is Dr. Herbert Wiser and the co-project officer, Dr. Morris Kelsey. Dr. Sober.

PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Dosimetry Studies in Selected Locations Where Epidemiology Studies Are Planned

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ABSTRACT

MASSACHUSETTS GENERAL HOSPITAL
EPA GRANT #R807222-01

UV-B DOSIMETRY STUDIES UTILIZING AN ELECTRONIC DOSIMETER

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Origins and Current Status: Six personal ultraviolet B-sensitive electronic dosimeters developed by Photometrics-Boston College were subjected to a series of laboratory tests designed to study spectral response, dynamic range, spatial response, linearity, and effect of three environmental factors--temperature, humidity, and vibration. These dosimeters, which share the identical sensor fluor, $MgWO_4$, with the worldwide distributed Robertson-Berger meter had a peak response at approximately 300 nm with the bulk of response falling in the 280-320 nm range. Linearity of response was acceptable from 0.1 to 2.0 solar constants. 2/6 units tended to jam at high flux rates. Less than a 2.5% average variation was detected from 0% to 100% humidity. An acceptable temperature change of 0.5%/°C was noted from 0°C to 60°C. Some evidence of fluor deterioration was noted following vibrational testing. Absolute calibration differed between the units. Recalibration is essential before field trials are undertaken. Under normal indoor conditions, no UV-B is recorded.

Plans for Future Work: Arrangements have been made for the static and dynamic outdoor field trials of the electronic dosimeter on Cape Cod, July-August, 1981. Laboratory testing will have been completed before these trials commence. In order to dynamically test the electronic dosimeters, special metal holders were fabricated by the Massachusetts General Hospital's machine shop. The dosimeter is carried in a metal cradle which in turn is attached with a bulldog clip to the test subject's pocket or belt. Since the electronic dosimeters have an approximate depth of one centimeter, the units protrude above the surface of the subject. Problems with the present system include: 1) inability to place the dosimeter on certain body prominences such as the head, neck, or shoulders (areas of major sun exposure); and 2) some mobility of the unit occurs so that the detector window is not always parallel with the skin surface. A possible solution would include the use of small lightweight sensors that could be developed to attach to exposed surfaces which could relay information to a larger recording device attached on belt or pocket.

Acknowledgement:
This research is supported by NCI/EPA joint environmental cancer studies.

Our mission has been to evaluate ultraviolet personal dosimeters for the EPA. The genesis of this project resulted from a recommendation of the National Academy of Sciences to EPA that personal ultraviolet dosimeters be developed so that objective measurement of the dosage of ultraviolet light that individuals were receiving could be obtained. Depending purely on personal recall in epidemiologic studies for determination of previous exposures is hazardous because of faulty memory.

Why are we doing this kind of work at all? Non-melanoma skin cancers in the United States by NCI estimates are now approximately one-half million per year. When one considers their anatomic distribution, what is seen is that for basal cell carcinoma, the most common type of skin cancer, over 90 percent of these lesions occur on the head and neck, with less than 10 percent occurring over the rest of the body. This pattern fits a sun-exposed pattern quite nicely.

When we consider, however, malignant melanoma, you don't see that type of distribution.(Fig. 1) Yet there is substantial epidemiologic evidence suggesting that sunlight is a factor in the genesis of malignant melanoma as well.

Rather than cumulative dose-exposure in melanoma, it may be short-term acute sun exposure. There is,therefore, an interest in trying to determine sun exposure from recreational travels;i.e. sun exposure that one might obtain in winter from skiing; and occupational sun exposures, as well as long-term profiles of individual sun exposure.

In response to this need the EPA issued a contract to a group at Boston College and Photometrics Laboratories in

Lexington. This group's mission was to develop an electronic ultraviolet B dosimeter.

Figure 2 is a picture of the unit that they developed which is a prototype model. There are six in existence. The device has the identical sensor as the Robertson-Berger meter which is in widespread use around the United States and at certain sites around the world.

Basically, this device works by employing a magnesium tungstate fluor beneath a filter that allows passage of ultraviolet B. The fluor responds to the UVB with the emission of a photon of light which is recorded on a photo cell and then through a fairly complicated electronic circuitry, a digitized recording is made so that the unit will read out in a digital manner.

This device is proported to be calibrated so that it reads the same as the Robertson-Berger meter. We have not evaluated the calibration because we don't at present have a Robertson-Berger meter in Boston. We plan soon to purchase an R-B meter and set it up in Boston and at that time we will be able to check the calibration of this unit against the Robertson-Berger meter.

The prototype electronic dosimeter has appreciable thickness, as well as significant size. It is 4.7 centimeters by 1.5 centimeters, and weighs 60 grams which, for non-metric people, is about two ounces. 440 of the digital counts is equal to what is termed one sunburn unit, which is the amount of sun necessary to create minimal redness in a Caucasian.

Figure 3 represents data from Boston College showing the response of this meter in relationship to the erythema action spectrum. The curve on the left is the erythema action spectrum; the curves on the right (superimposed) are the Robertson-Berger meter and the personal dosimeter. The personal dosimeter does appear to measure what the Robertson-Berger meter measures,but there is a spectral shift toward the UVA range for both devices so that there is not an exact match to the erythema action spectrum.

Figure 4 is the angular sensitivity of the personal dosimeter as its face is altered in the direction of ultraviolet exposure. The dark line is the theoretical cosine curve for ideal recording, and the dash line is what this device actually records. Up to about 50 degrees incidence the device reads fairly close to what you would expect from a cosine curve but once you get beyond that, there are fair amounts of disparity between what the meter will read and what is actually incident.

We had six units for testing. The peak wave length of response on all units was approximately 300, which is right in the middle of the UVB range where you would want the meter reading. And the half peak wave length was about 10 degrees on either side. So we felt the unit reads in the UVB range where it is supposed to.

Next, we determined the linearity of the meters. We measured from 1/10 solar constant up to two solar constants and over this range the meters do read fairly linearly.

There was a problem, however, in that two of the meters would freeze at the higher flux rates, that is, between one to two solar constants, two of the six meters would jam and not read further, and would have to be unjammed by manipulation. For most measurements you would be below one solar constant so this jam up would not be a problem.

We next wanted to see whether the meters would read identically to the same flux exposures. Table 1 is the raw data on the six meters. The counts range over the time periods studied, from 1204 counts to 1744. This was a little disturbing since there was a difference of about 50 percent between these meters. The meters would always read out in the same order so it looked as if it was a calibration problem and not a problem that was inherent in the meters. After our testing was completed in the laboratory,we sent the meters back to Boston College for recalibration and standardization before the field trials.

With humidity testing, there was less than 2.5 percent variation between zero percent humidity and 100 percent humidity, which we felt was quite acceptable. With temperature testing there was 0.5% change per degree centigrade. There is a typographical error in the meeting abstract which says that temperature change was unacceptable. We feel the temperature change was certainly acceptable, a half a percent per degree is not really a problem.

We next began outdoor field testing in which we exposed the meters to direct sunlight and measured the counts hourly. Figure 5 shows that peak counts occur around noon with a fall off on both sides. If you record the counts from 10:40 to 2:40 in the afternoon and integrate that,what you find is that you have two-thirds of the ultraviolet light coming down from 10:40 to 2:40, which fits the generally accepted notion about 70 percent of the day's ultraviolet occurring in that time period. The curve nicely falls as it gets later in the day, just as expected.

We also tested the unit to see whether it would record underneath a white cotton shirt because we were curious why there were so many melanomas occurring on the back. About eight percent of the incident ultraviolet will pass through a white cotton T shirt. In addition, we were interested in the site variation on the body. These units were put on the top of the head on a hat, and also allowed to hang from a belt at waist level. The reading on the belt is about 18 percent of the reading on the cap, which also fits with the notion of direct incident light occurring on the top of the head versus the side of the body.

During the field trials only four of the six units were operational. The other two units would not record. These two were returned at the end of the trial to Boston College for overhaul. The other four units - all of which had been recalibrated - three of the four read within five percent of each other, and the fourth one read about 10 percent below the others.

We were to comparison test these electronic dosimeters against film badged dosimeters supplied by the EPA. These have been slow in coming, but in the meantime Boston College created some polysulfone film badges for us to test. Five and ten percent polysulfone film badges when exposed to increasing flux will read fairly linearly. Since the polysulfone film badges are shifted to UVA and do not match well with the erythema action spectrum, we discontinued looking at them at Dr. Wiser's suggestion.

We are now planning on testing a photographic film dosimeter to be developed by Dr. Urbach at Temple through an EPA contract and a commercial dosimeter that is available from 3M. The 3M film dosimeter is based on a photochromic reaction. The dye, when exposed to energy, changes color. The spectral response is from 280 to 320nm . The rest of the ultraviolet and visible wavelengths are blocked out by a filter.

This dosimeter is commercially available. It comes free with a bottle of suntan lotion marketed by a 3M subsidiary. The purchasers can use this meter to see how much sun they are getting so they know how long to stay out in the sun based on their sun type and the degree of photo protection from the sun blocker.

Our plan is to try this device in the lab and field trial against the electronic one and, hopefully, Dr. Urbach's photographic film dosimeter as well.

Figure 1

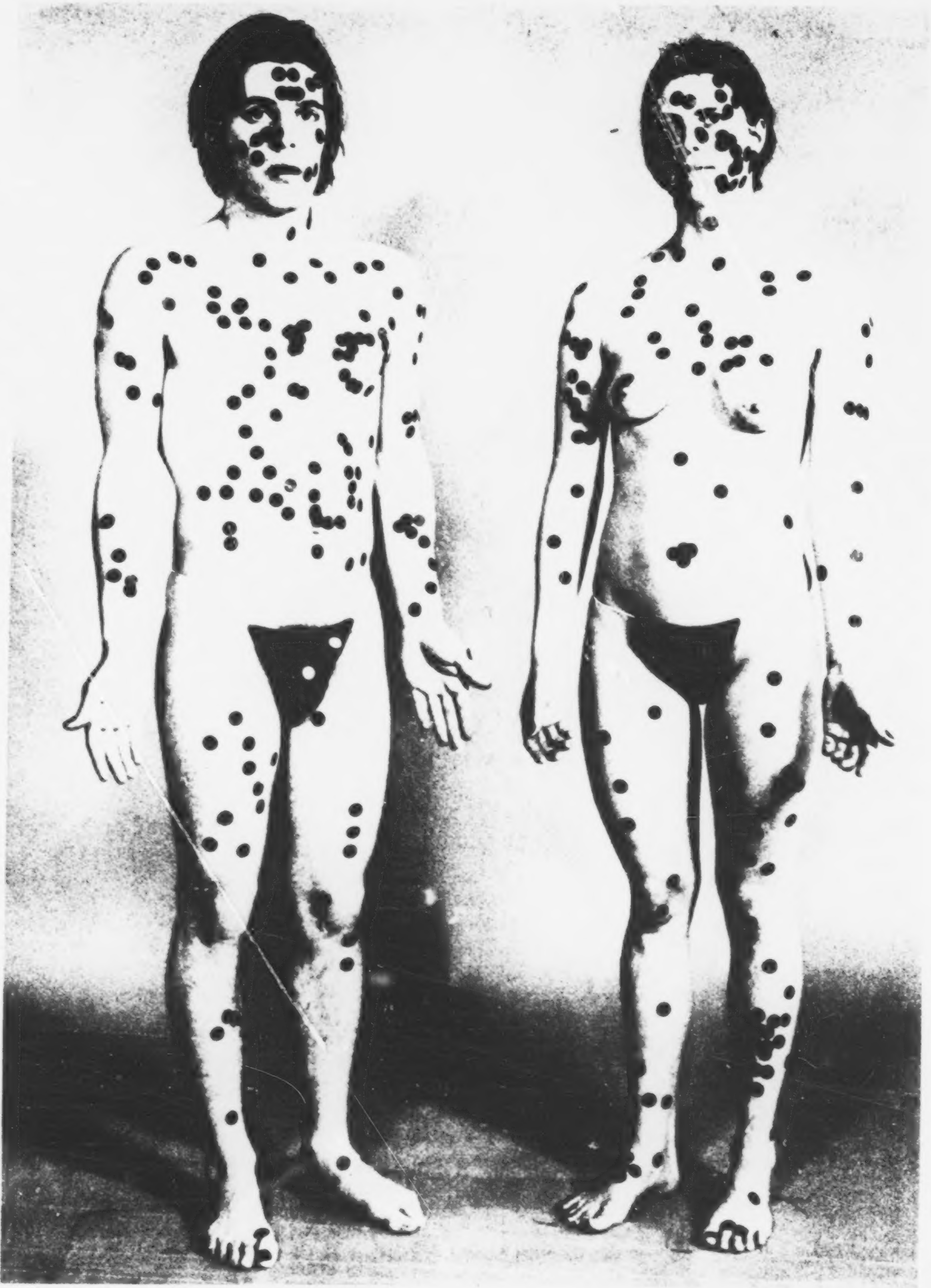
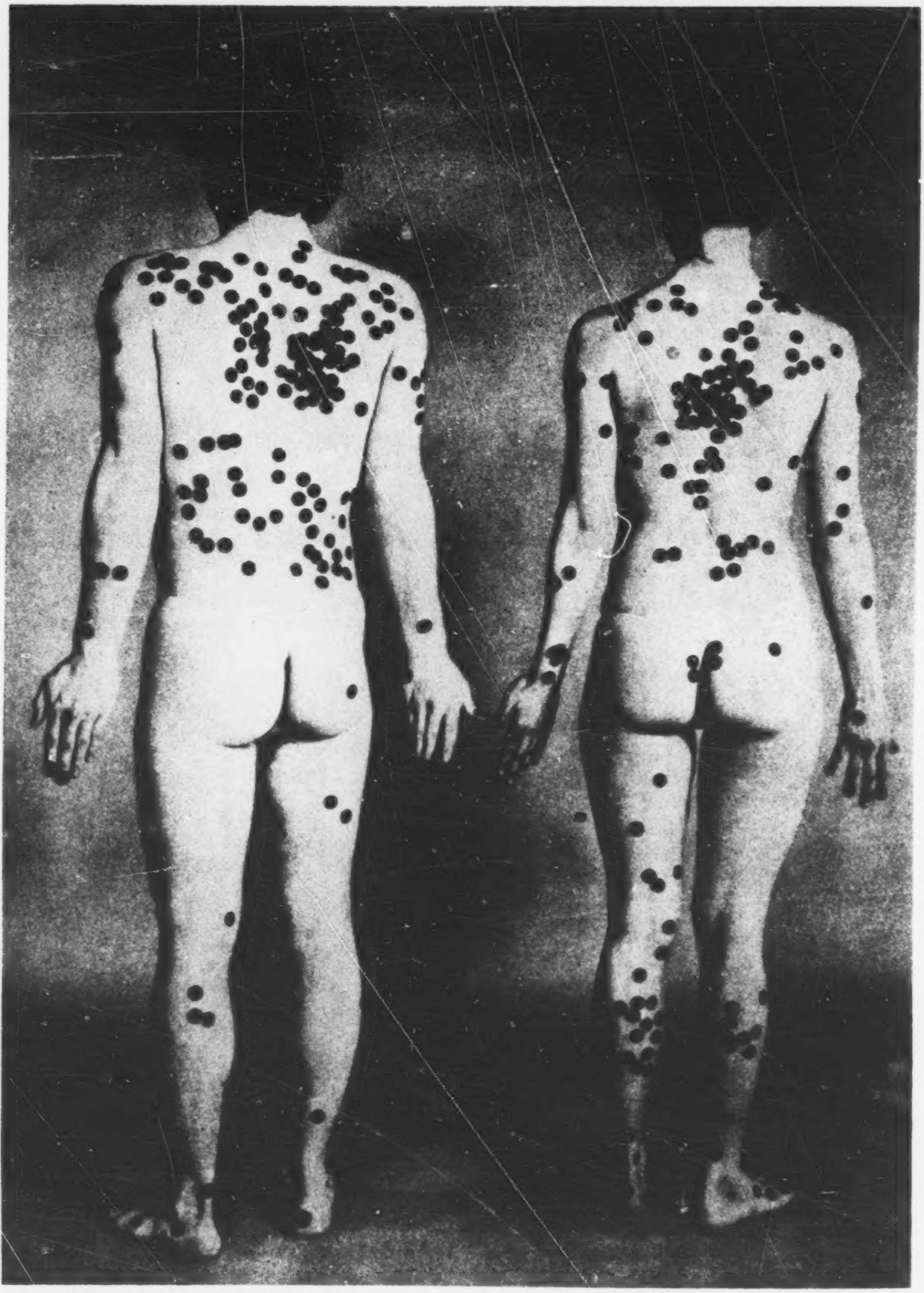


Figure 1



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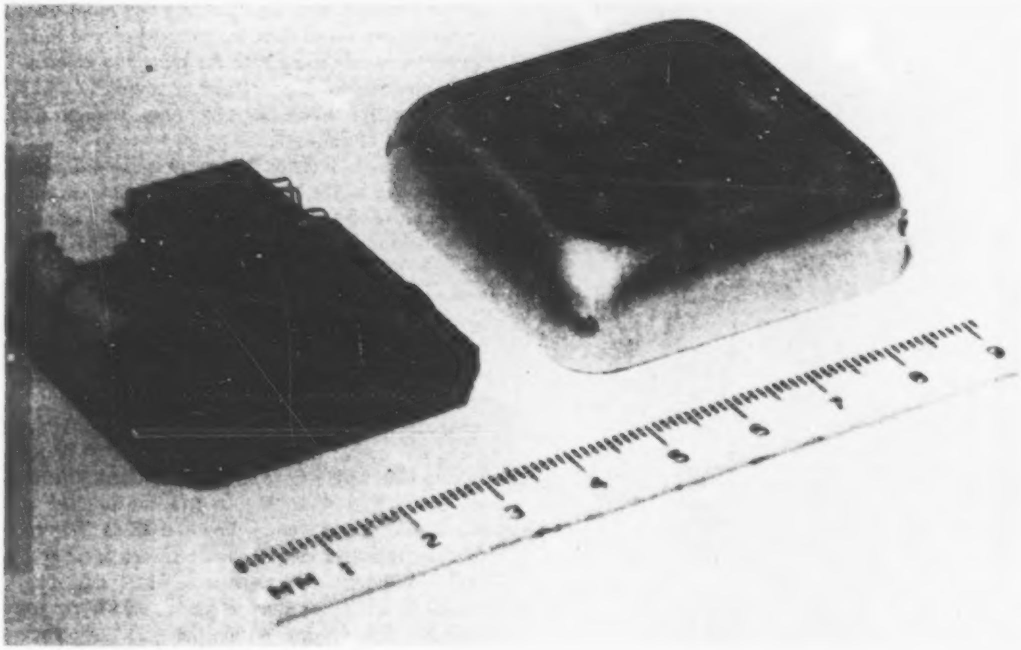
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Figure 2

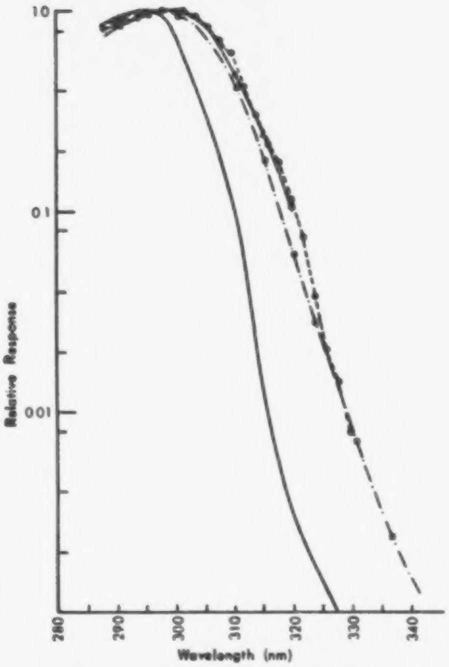


Figure 3. Spectral response of the UV-B personnel dosimeter (●--●), and of a Robertson–Berger sunburning UV meter measured in the same optical system (▲——▲) and as reported by Berger (1976) (■-·-■). The erythemal action spectrum (Berger, 1976) (————), also normalized at 297 nm, is shown for comparison.

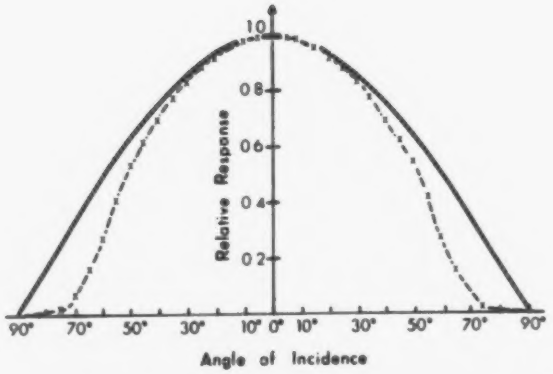


Figure 4 Angular sensitivity of the UV-B personnel dosimeter as measured with a collimated beam from a deuterium lamp (× -- ×), and "ideal" cosine response (————).

Table 1

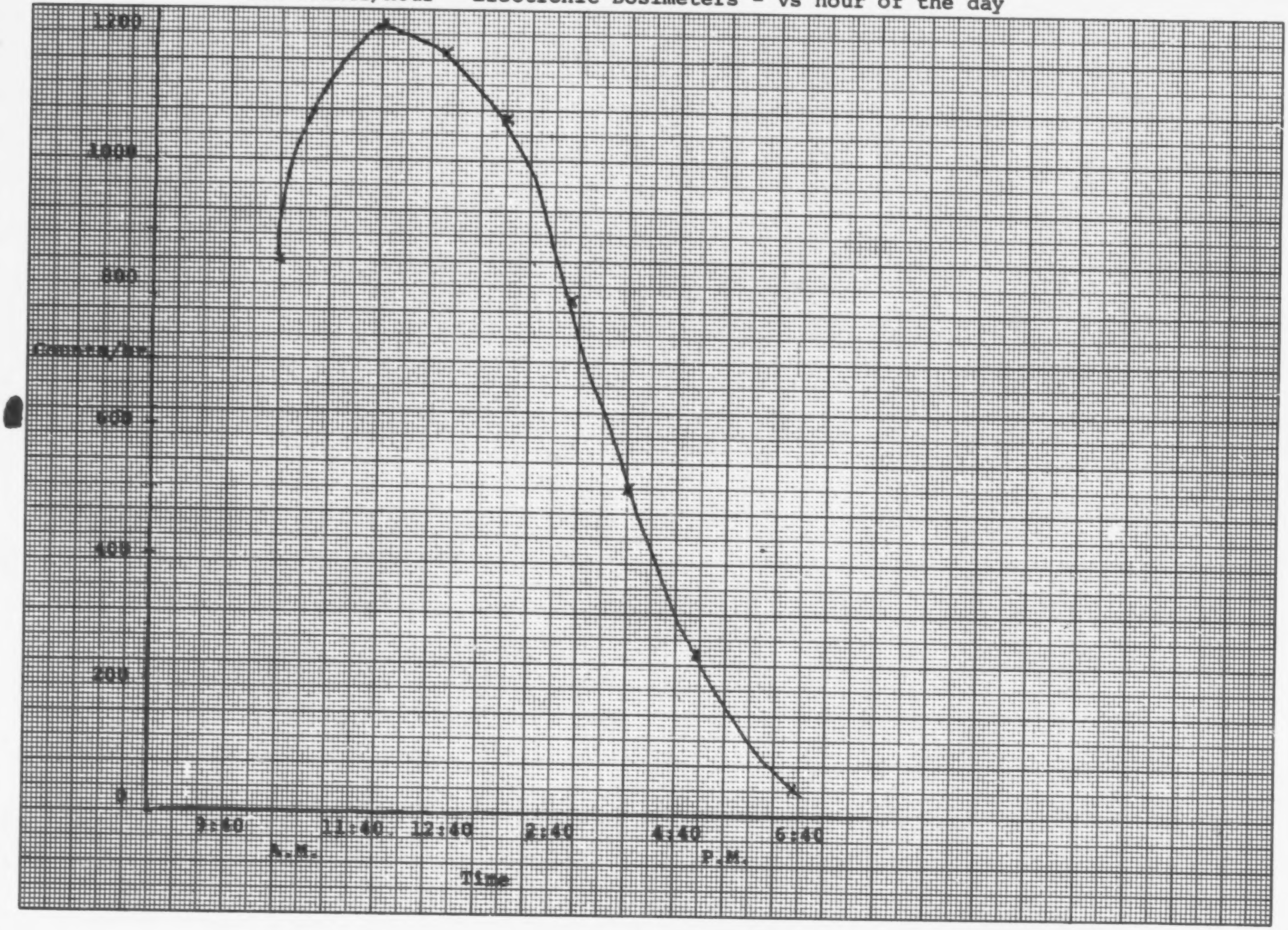
Variation in Meter Readings After Identical UVB Exposure†

Unit #	Trial 2	Trial 2
1	1360	1340
3	1544	1612
4	1244	1256
5	1204	1260
6	1560	1560
7	1744	1692

†20 minutes at 1 solar constant

FIGURE 5

Mean Counts/hour - Electronic Dosimeters - vs hour of the day



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PROCEEDINGS OF THE
SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Cocarcinogenicity of Foundry Particulates

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COCARCINOGENICITY OF FOUNDRY PARTICULATES--INTERIM REPORT

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ABSTRACT

The traditional use of silica sand for metalcasting is slowly being replaced by non-silica aggregates due to increased demands for improved cast surfaces and closer dimensional tolerances, and to the physical, chemical and biological limitations of silica sands. The major non-silica aggregates presently used throughout the metalcasting world include zircon sand, olivine sand, chromite sand and aluminum silicates. However, little is known about the biological effects of these materials.

The sands are currently being evaluated for both fibrogenic and cocarcinogenic potential in the hamster by intratracheal administration of the commercial sands with or without concurrent administration of benzo(a)pyrene (BaP). Respirable fractions of the sands were prepared either by sieving and air classification or by micronizing. Particle size analyses of the respirable fractions showed that more than 99% of the particles of each sample were less than 10 μm and that surface areas varied from 1.01 to 2.41 m^2/g. Free silica contents of the non-siliseous sands were less than 1%. Chrysotile asbestos fibers were found in both olivine and chromite sand samples and amphibole fibers were found in the latter.

The study is designed so that remaining hamsters will be killed 90 weeks from the start of exposure. Interim results, reported here, show that lung tumors have been found in all groups given the aggregate plus B(a)P, and that no lung tumors have been found in the absence of B(a)P.

The relative severity of the fibrogenic and carcinogenic responses must await final histopathology and statistical analyses.

Acknowledgement:

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INTRODUCTION

In the U.S., in 1978, there were over 400,000 production workers employed by more than 4,400 foundries. Ferrous type castings accounted for 85% of the total castings produced by these foundries; the remaining castings were aluminum (10%), zinc (2%), copper (2%) and magnesium (1%) (Palmer and Scott, 1981).

The foundry process basically involves the following steps: melting and alloying the metal; preparing the sand mold and cores; pouring of the molten metal into the mold; cooling of the mold and castings; shakeout, where the hot castings are separated from the molds; and finishing by blasting and grinding the castings with abrasives.

The molds used for metal casting are usually composed of silica sand. However, the tradition of using silica sand for metalcasting is slowly being changed to the use of non-silica aggregates because of increased demands for improved cast surfaces and closer dimensional tolerances, and because of the physical, chemical and biological limitations of silica sands.

The performance of some non-silica mold/core materials had been evaluated as early as the 1920s. However, their use was not established until casting designs became more complex and producers could supply aggregates of more uniform and consistent quality. Early use of these non-silica sands was investigated primarily for their characteristics and properties at elevated temperatures. Wettability by steel, thermal expansion, heat diffusitivity, refractory characteristics and cost are the major factors when considering their use (Kotzin, 1981). The Scandinavian countries have added one more consideration to the list. They reported that silica sand substitutes, such as olivine, may reduce some adverse biological effects such as silicosis, and routine use of olivine in place of silica sand was established in the 1940's in these countries (Tubich, 1967).

The major non-silica aggregates presently used throughout the metalcasting world include zircon sand, olivine sand, chromite sand and aluminum silicates (Kotzin, 1981). However, little is known about the biological effects of these materials.

Recent epidemiological studies have concluded that there is a significant excess of lung cancer, as well as nonmalignant respiratory disease among foundry workers (Egan, et al., 1977). Palmer and Scott (1981) in their review article addressing the cancer problem, discussed the possible agents responsible for contributing the hazard. These included various classes of carcinogens such as polynuclear aromatic hydrocarbons, metals and other organic compounds, cigarette smoking, and promoters/cocarcinogens, such as irritants and particulates.

The cocarcinogenic effect of particulates has been well established by use of the intratracheal instillation model for studying lung cocarcinogenesis. Ferric oxide (Saffiotti, 1968), carbon (Farrell and Davis, 1972), titanium dioxide (Stenback, et al., 1976), magnesium oxide (Stenback, et al., 1975), and amorphous silica (Stenback and Rowland, 1979) in combination with the ubiquitous carcinogen, benzo(a)pyrene (B(a)P) significantly increase the number of pulmonary tumors, compared to B(a)P alone. However, the results with aluminum oxide (Farrell and Davis, 1972; Stenback, et al., 1976) and manganese oxide (Stenback and Rowland, 1979) do not show a cocarcinogenic effect. Since the cocarcinogenic effect of particulates in combination with B(a)P administration cannot be generalized, it is necessary to study each material.

This study was designed to focus on both aspects of the pulmonary pathology experienced by foundry workers, silicosis and cancer as caused or modified by foundry aggregates. The purpose of this investigation was threefold: first, to assess the cocarcinogenic potential of silica sand; secondly, to assess in the animal model the cocarcinogenic potential of silica sand substitutes including, olivine from 2 geographical sources, chromite sand, zirconium silicates from 2 geographical sources and aluminum silicates of 3 types; and thirdly, to assess the relative fibrogenic activity of these materials as compared to a reference quartz (Minusil).

MATERIALS AND METHODS

Prior to the beginning of the animal study, selected physical and chemical parameters for the various particulate materials to be used were evaluated. The analyses performed for each material included particle size, surface area, fiber counts, free silica and chemical content of bulk samples and of 1,000 individual particles. The results of these physical and chemical analyses were reported previously (Stettler, et al., 1981).

A total of 10 sands were characterized. Nine of these sands are used in foundries; the tenth, Minusil glass sand, was used as a positive control for silicosis in the animal study. A list of the sands and their suppliers is given in Table 1. The nine foundry sands, as commonly used in foundries, required processing because only the respirable fractions were desired for the animal experiments. To accomplish this for three of the materials, commercially available flours (items 11-13, Table 1) were used as the starting material. The Minusil sample was used as received from the supplier.

A standard 325 mesh, 30-inch stainless steel screen was used to eliminate the oversize (+44 μm) fraction of the coarse materials. A combination of dry sieving and air classification was used as a method of preparing the respirable fractions of the aluminum silicate sands. An air classifier (Donaldson Co., Model B-18 Acucut) was used to prepare the respirable fractions from the -325 mesh fractions. Because this classifier required a minimum of 5 kg of starting material, air classification was performed only for the aluminum silicate sands. The -325 mesh fraction for the other sands was much less than 5 kg as can be seen from the data in Table 1. Consequently, where possible, commercial "flour" samples (-325 mesh) were used instead of the bulk samples to prepare the respirable fractions. Commercial flour samples were available for the Ottawa silica sand and the two zirconium silicate sands. Respirable fractions of these three materials were also prepared with the Donaldson air classifier. No flour samples were commercially available for the olivine samples and the chromite sand. Therefore, samples of the coarse material for each of these sands were micronized in a Trost mill (Garlock, Inc., Model Gem T) to prepare the respirable particulates. The Trost mill used in this operation had a polyurethane liner to minimize metal contamination.

Stock suspensions of the respirable fractions of the nine foundry sands and the Minusil glass sand were prepared as described by Stettler, et al. (1981) and aliquots were used to prepare duplicate filters for particle and fiber analyses. Samples were also prepared from the bulk coarse sands and bulk flours so that any changes in chemical composition imparted to the processed respirable particulates would be known.

For particle sizing and individual particle chemical analysis of the processed materials, one of the filters was examined directly with a scanning electron microscope equipped with an energy dispersive x-ray spectrometer system and an image analysis system using the back-scattered electron image.

The second set of filters, plus the blank filters and filters for the bulk coarse and flour sands, were used for fiber analysis using a transmission electron microscope equipped with an energy dispersive spectrometer system. Fibers from each sample were counted, sized, and then analyzed by energy dispersive x-ray analysis (EDXRA) and selected area electron diffraction (SAED).

Surface area determinations for each of the respirable sand fractions were performed by nitrogen adsorption using an automatic surface area analyzer.

Bulk chemical analyses were performed on aqua regia digests of each of the nine respirable dusts plus the Minusil sample. Analysis by atomic absorption spectrometry (equipped with a graphite furnace) was performed on each sample for beryllium, sodium, magnesium, and aluminum. Analysis for all elements heavier than aluminum (silicon through uranium) was performed simultaneously by proton-induced x-ray emission spectrometry (PIXE). All analyses were performed in duplicate. Radon daughter concentrations were determined (Sensintaffar, 1980) using bulk samples of the coarse zircon sands (Items 5 and 6--Table 1).

The respirable fractions of the non-siliceous foundry sands were analyzed for free silica using x-ray powder diffraction (XRD). Interferences were encountered with the three aluminum silicate samples. These samples were treated with a phosphoric acid digestion technique to eliminate the interferences and then reanalyzed by XRD.

The benzo(a)pyrene (Aldrich, Gold Label) was prepared in saline suspension by dry grinding in a mullite mortar for 24 hours and then fresh aliquots were prepared weekly in saline suspension by the technique of ball milling for 7 days. The suspension was stored in the dark at 5^o C during the week. Light microscopy methods were used to estimate B(a)P particle size distribution.

After a 2 week quarantine period, acceptable 6 to 8 week old male Syrian Golden Hamsters were randomly assigned to the treatment groups by computer generated random numbers. Treatment groups, consisting of 50 hamsters each, were given either the particulate alone or in combination with 3 mg B(a)P (Table 2). The suspensions were administered by intratracheal instillation according to the method of Saffiotti, et al. (1968). An instillation volume of 0.2 ml was used and the suspensions were kept homogeneous by magnetic stirring during the course of the instillation. The dose of each material (Table 2) was determined from the surface area data (Table 3) based upon the estimated maximum tolerated dose of Minusil taken as unity. For example, the estimated tolerated dose of Minusil (0.7 mg) would have a total surface area of 0.0021 m^2 (0.0007g x 2.99 m^2/g), and a comparable dose of chromite sand with a surface area of 1.01 m^2/g would be 0.0021 $m^2 \div 1.01$ m^2/g = 2.1g. The treatment schedule involved 15 weekly instillations under Sodium Brevital anesthesia. For positive controls, ferric oxide and B(a)P were used for cocarcinogenesis and Minusil for fibrogenesis. The ferric oxide plus Minusil group was included to test the combined effect. In addition, a ferric oxide alone and saline alone were used as negative controls and B(a)P in saline was used as an additional control.

Hamsters are housed individually in polycarbonate cages containing sterile San-i-cel, suspended from stainless steel racks. Cages are washed weekly. Water and certified food are provided ad libitum. Mortality and morbidity observations are made twice daily and pharmacotoxic/clinical signs of illness are made once weekly. Body weights were taken weekly during the first 15 weeks of treatment and at two-week intervals thereafter.

Surviving animals will be killed in January 1982 at 21 months after the initial treatment. The standard National Cancer Institute's protocol will be used for necropsy and histopathological evaluation. Fibrogenic and tumorigenic responses will be emphasized in the examination of the lung. All examinations will be made by or under the supervision of a Board Certified Pathologist with at least five years experience in experimental tumor pathology. Animal weights, latent periods, times of death, number, types and locations of tumors, degree of fibrosis, etc., will be recorded and statistically analyzed.

RESULTS

The results of the particle size and surface area analyses for the 10 samples are summarized in Table 3 and the particle size distribution curves are given in Figures 1-4.

The major difference between particle distribution obtained by air classification and micronizing is readily apparent in both the particle size distributions and electron micrographs. Air classification produced very narrow size distributions, whereas micronizing produced much broader distributions. Micronizing produced lower median diameters (0.41-1.31 μm) than air classification (1.95-2.62 μm). This reflects the greater percentages of submicron particles present in the micronized samples.

The concentrations of the trace elements found in the respirable fractions of the 10 samples and the free silica contents of these materials are summarized in Tables 4 and 5. Except for the Ottawa sand and the Minusil sand, which are essentially quartz, the sands contained less than 1% free silica. Elemental concentrations of particular interest are the high chromium (2,030-2,500 μg/g) and nickel (2,100-2,400 μg/g) concentrations found in the olivine samples and the high hafnium concentrations (6000–7000 μg/g) of the zircon sands. The zircon sands contained total radionuclide concentrations, which are associated with radon and radon daughter decay schemes, of 202.7 and 163.1 pCi/g for the Florida and Australian sands, respectively.

The results of the individual particle analyses by SEM-EDXRA-IA are given in Tables 6 and 7. Table 6 shows the number of times a particular element was detected in these analyses. The various elemental combinations found within the particles are shown in Table 7. Because oxygen is not detected by the energy dispersive spectrometer, particles containing only silicon, aluminum, titanium, or iron are presumed to be oxides. The "Other Oxide" category in Table 7 is composed of particles containing varying combinations of magnesium, aluminum, titanium, or iron. The "Other Silicates" and "Other Aluminum Silicates" categories are composed of particles containing various combinations of sodium, magnesium, potassium, calcium, titanium, and iron in addition to major peaks for aluminum and/or silicon. The "No X-rays" category is for particles in which no elements were detected. These particles may be composed of elements not analyzed or more likely, organic particles. Most of the particles in the "Miscellaneous" class appeared to be composite particles, that is, particles composed of the elements found in two or more of the major particle classes. One exception to this was in the chromite sand. The three particles in the miscellaneous category for this sample were composed of major amounts of iron, copper, and titanium.

The results of the fiber analyses for the respirable fractions of the 10 samples, as well as for the original materials used in making these preparations, and for six blank preparations are given in Table 8. A fiber was defined as any particle with an aspect ratio greater than 3:1. Detailed results of fiber analyses were reported previously (Stettler et al., 1981). All of the fibers found in the olivine samples were magnesium silicates, and some of them contained small amounts of iron; approximately 58% had diffraction patterns typical of chrysotile asbestos, and the remaining 42% had either ambiguous SAED patterns or gave no patterns.

The bulk chromite sand contained two different types of fibers. Fifty percent had energy dispersive x-ray spectra typical of chrysotile asbestos, that is, the fibers were magnesium silicates, some containing small amounts of iron. The remaining fibers had peaks for calcium, magnesium, iron and silicon in their x-ray spectra. The elemental make-up of these fibers and their SAED patterns indicate amphiboles belonging to the tremolite-actinolite series.

Particle size distribution of the B(a)P in saline solution was found to be the following: <10 μm--80%; <9 μm--75%; <8 μm--70%; <7 μm--64%; <6 μm--58%; <5 μm--50; <4 μm--40%; <3 μm--30%; <2 μm--19%; <1 μm--7%.

Animal Experiments

Results based upon gross observations after 15 months of the scheduled 21 month study are summarized in figures 5-10. Survival of the saline control group has decreased to about 70% at 15 months. Except for American Zircon and Washington olivine sands with survival rates of 56 and 54%, respectively, at 15 months, groups given particulates alone have survival rates similar to the control and range from 84 down to 62%.

Survival of the particulate plus B(a)P groups range from 68 down to 32% and are in all cases lower than their respective controls. Relative survival rates of the particulate plus B(a)P groups compared to their respective controls vary from 50%

(Australian zircon sand) to 97% (Saline control).

Lung tumor development, based upon gross observations and defined as unusual macroscopic tissue masses, appears to have affected survival. Gross lung tumor data collected at necropsy show current tumor incidence based on the original number of animals ranging from 14 to 36% in the particulate groups. The current tumor incidence of the positive control group (Fe_2O_3 plus B(a)P) is 28%. Lung tumors have been observed in every group receiving particulate and B(a)P. Gross lung tumor incidence in the B(a)P saline control group is only 6% after 15 months. No evidence of gross tumor development has been seen in any of the groups receiving particulate alone. Histopathological diagnosis and confirmation of the gross observations are in progress.

SUMMARY AND CONCLUSIONS

The first objective of this study was to prepare respirable size fractions of nine foundry sands to be studied in the subsequent animal exposure experiment. The original intent was to separate these respirable fractions from the coarse sands that are used in foundries by a combination of sieving and air classification. This did not prove practical for all samples because of the small weight percentages of respirable particles present in some of the test materials. Consequently, air classification of flour samples for some of the materials and micronizing of coarse sands of others was necessary to produce the quantities of test materials needed. However, the final products obtained for all of the nine foundry sands do represent almost completely respirable fractions. The number median diameters for those preparations range from 0.41 to 2.62 μm. Greater than 99% of the particles for each preparation have diameters less than 10 μm. The surface areas of the nine preparations range from 1.01 to 2.41 m^2/g.

A brief summary of those conclusions of the particle analyses is given here; they have been extensively discussed by Stettler et al., (1981).

Olivine is a solid solution of magnesium silicate (fosterite) and iron silicate (fayalite) whose composition is $(Mg-Fe)_2SiO_4$. Over 13% of the North Carolina olivine particles appeared to be fosterite as compared to only 1% found in Washington sand. Approximately 93% of particles were composed of these two types. Free silica content of both samples was less than 0.5%. Nickel was found as a trace component which was evenly distributed, while chromium occurred as a principal component of a few particles. Chrysotile asbestos fibers were found in both the North Carolina and Washington olivine samples. These fibers were typically very thin with diameters less than 0.3 µm. The aspect ratios for these fibers were usually much greater than 3:1, typically on the order of 10:1 and greater. The presence of chrysotile asbestos in olivine has been reported before (Kuryvial et al, 1974).

In the characterization of the zircon sands, ZrSiO4, zirconium and silicon were the only major components comprising 94.8% of the samples from both geographical sources. The free silica content was less than 0.5%. Titanium, iron, yttrium and hafnium were significant trace elements found in both samples; the latter was distributed throughout the particles. The implications of the abnormally high radon daughter levels have been discussed by Boothe et al. (1980). The independent findings of Boothe and coworkers has resulted in the Oregon State Health Division imposing limited controls on the radiological aspects of foundry operations where major foundries have been required to obtain radioactive materials licenses to use and dispose of zircon sands.

In the individual particle analyses of chromite sand, $Cr_2O_3 \cdot FeO$, chromium and iron occurred together as the major components of over 94.6% of the particles. Aluminum and magnesium, known minor constituents of this sand (Goethman, 1975), were found in the bulk samples at concentrations of 0.7 and 1.4%, respectively. Free silica content was found to be less than 0.5%. Small numbers of chrysotile asbestos fibers and fibers that appear to be amphiboles of the tremolite-actinolite series were found in the chromite sand.

The major particle type found for the three aluminum silicate samples contained only aluminum and silicon comprising 94 to 96% of the particles. The ratio of aluminum to silicon was approximately 1:1 for Remasil 48, 1.5:1 for Remasil 60 and 3.9:1 for Remull. The principal contaminant was aluminum, presumably aluminum oxide, ranging from 2.33 to over 10.3%. Free silica content ranged from 0.6 to 1.8%.

Particles containing only silicon, presumably silica, were the major components of both the Ottawa sand (97.7%) and the Minusil sand (96.9%). X-ray diffraction analyses confirmed that both of these samples were primarily crystalline silica (quartz). The principal contaminants found in both samples were particles considered iron oxide and various silicates and aluminum silicates.

Interpretation of the data from the intratracheal instillation studies must await complete histological examination and statistical analyses.

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Mention of commercial products in this paper does not indicate endorsement by the National Institute for Occupational Safety and Health.

Table 1. Sand Materials and Their Sources

	Materials	Sources	Starting Weights (kg)	-325 Fraction (kg)
1.	Ottawa Silica Sand, GFN* 56	Michigan Terrazo 12900 Evergreen Road Detroit, Michigan 45223	227	0.005
2.	Chromite Sand, GFN 55/60 Manufacturer: American Colloid	Carpenter Brothers, Inc. 606 W. Wisconsin Avenue Milwaukee, Wisconsin 53203	227	0.68
3.	Olivine Sand (North Carolina), GFN 70	IMC Chemicals Group, Inc. P. O. Box 207 Terre Haute, Indiana 47808	227	Trace
4.	Olivine Sand (Washington), GFN 70	IMC Chemicals Group, Inc. P. O. Box 207 Terre Haute, Indiana 47808	227	0.227
5.	Zirconium Silicate Sand (Florida), GFN 94-100, Calcined	Continental Mineral Processing Co. 11817 Mosteller Road Cincinnati, Ohio 45241	227	0.01
6.	Zirconium Silicate Sand (Australia), GFN 94-100, Non-calcined	Continental Mineral Processing Co. 11817 Mosteller Road Cincinnati, Ohio 45241	227	0.01
7.	Aluminum Silicate Sand, Remasil 48 RP 200C	Remet Corporation P. O. Box 278 Chadwick, New York 11319	136	49
8.	Aluminum Silicate Sand, Remasil 60 RP 200C	Remet Corporation P. O. Box 278 Chadwick, New York 11319	136	65.3
9.	Aluminum Silicate Sand, Remull RP 325, Coarse Grind	Remet Corporation P. O. Box 278 Chadwick, New York 11319	136	27.2
10.	Control Silica Sand, Min-U-Sil, 5 µm	Pennsylvania Glass Sand Corporation 3 Penn Center Pittsburg, Pennsylvania 15235	2.2	2.2
11.	Ottawa Silica Sand, Sil-Co-Sil 395, -325 GFN	Ottawa Silica Company P. O. Box 577 Ottawa, Illinois 61350	136	136
12.	Zirconium Silicate Sand (Florida), -325 GFN, Calcined	Continental Mineral Processing Co. 11817 Mosteller Road Cincinnati, Ohio 45241	136	136
13.	Zirconium Silicate Sand (Australia), -325 GFN, Non-calcined	Continental Mineral Processing Co. 11817 Mosteller Road Cincinnati, Ohio 45241	136	136

From Stettler et al., 1981

*GFN is the grain fineness number and is synonymous with mesh size

Table 2. Treatment Groups and Dosage[a] of Particulates

Group No.[c]	Treatment	Dosage of Particulates
1	Vehicle Control (Saline)	none
2	Vehicle Control + B(a)P[b]	none
3	Ferric Oxide	3 mg
4	Ferric Oxide + B(a)P	3 mg
5	Silica Sand-Ottawa	1.1 mg
6	Silica Sand-Ottawa + B(a)P	1.1 mg
7	Minusil	0.7 mg
8	Minusil + B(a)P	0.7 mg
9	Zirconium Sand - Australian	0.9 mg
10	Zircon Sand - Australian + B(a)P	0.9 mg
11	Zircon Sand - American	0.9 mg
12	Zircon Sand - American + B(a)P	0.9 mg
13	Chromite Sand	2.1 mg
14	Chromite Sand + B(a)P	2.1 mg
15	Minusil + Ferric Oxide	0.7 mg + 3 mg
16	Minusil + Ferric Oxide + B(a)P	0.7 mg + 3 mg
17	Olivine Sand (NC)	2.1 mg
18	Olivine Sand (NC) + B(a)P	2.1 mg
19	Olivine Sand (WA)	1.2 mg
20	Olivine Sand (WA) + B(a)P	1.2 mg
	Aluminum silicates:	
21	Remasil - 48	1.4 mg
22	Remasil - 48 + B(a)P	1.4 mg
23	Remasil - 60	1.4 mg
24	Remasil - 60 + B(a)P	1.4 mg
25	Remull RP 325	1.9 mg
26	Remull RP 325 + B(A)P	1.9 mg

[a] all doses instilled in 0.2 ml saline

[b] all B(a)P groups instilled with 3 mg B(a)P plus amount of particulate as shown

[c] group size = 50 hamsters

Table 3. Particle Size and Surface Area Analyses[g]

Sample	Specific Gravity	Median Diameter (μm)	Mass Median[e] Diameter (μm)	Mass Median[f] Aerodynamic Diameter (μm)	Surface Area (m^2/g)
Zircon Sand (Australia)	4.65	1.98	3.32	7.16	2.41
Zircon Sand (Florida)	4.65	1.95	3.11	6.70	2.26
Ottawa Sand	2.66	2.61	4.43	7.23	1.88
Chromite Sand	4.45	1.31	4.06	8.57	1.01
Olivine Sand (Washington)	3.30	0.98	4.05	7.36	1.71
Olivine Sand (North Carolina)	3.30	0.41	5.46	9.92	1.02
Remull RP 325[a]	2.85	2.36	3.46	5.84	1.11
Remasil 60[b]	2.78	2.62	3.79	6.72	1.46
Remasil 48[c]	2.65	2.60	4.52	7.35	1.47
Minusil	2.66	0.83	3.25	5.06	2.99
Ferric Oxide[d]	5.24	0.27	0.6	1.37	6.90

[a] Minerology - 87% mullite, 3% alumina, 10% glass
[b] Minerology - 80% mullite, 20% glass
[c] Minerology - 62% mullite, 38% cristobalite plus glass
[d] Fisher Scientific Co. (certified grade, red anhydrous--Lot 785156)
[e] calculated from circular equivalent diameter, assuming the particles were spheres
[f] calculated as Mass Median Diameter times the square root of the density
[g] modified from Stettler et al., 1981

Table 4. Bulk Chemical and Free Silica Analyses [1]

Element	North Carolina Olivine (µg/g)	Washington Olivine (µg/g)	Florida Zircon (µg/g)	Australia Zircon (µg/g)	Chromite Sand (µg/g)
Be		0.7±0.2			
Na		280±30			
Mg	Major	Major	80±20	70±20	14±2 (mg/g)
Al					7000±500
Si	Major	Major	Major	Major	
Ca	350±60	1800±200		600±200	
Ti		110±40	1800±200	3300±500	3000±200
V					1200±300
Cr	2500±100	2040±60		110±40	Major
Mn	600±100	900±100			2600±900
Fe	50.3±0.7 (mg/g)	60±10 (mg/g)	1200±100	1870±90	Major
Ni	2400±300	2100±200	51±10	70±10	540±50
Zn	16±3	45±10		30±10	450±30
As	1.7±0.8	3.8±0.5		110±20	
Cd	22±9	14±3			16±7
Br		7±1			38±4
Rb		1.7±0.5			
Sr		5.1±0.8	400±100	240±90	6±2
Ga					24±3
Zr			Major	Major	30±3
Se					810±90
Sc			210±70		
Y			2300±200	2000±400	
Hf			7000±200	6000±1000	
I				80±20	
Au				60±20	
U			500±100		
Free Silica (%)	<0.5	<0.5	<0.5	<0.5	<0.5

*Data presented are the average of two determinations with the standard deviation.

Table 5. Bulk Chemical and Free Silica Analyses [1]

Element	Remasil 48 (µg/g)	Remasil 60 (µg/g)	Remull RP 325 (µg/g)	Ottawa Sand (µg/g)	Min-U-Sil Sand (µg/g)
Be			0.4±0.2		
Na	90±40	210±50	100±30		
Mg	450±20	1040±50	510±30		180±20
Al	Major	Major	Major		
Si	Major	Major	Major	Major	Major
Ca	500±100	900±200	700±100	330±100	
Ti	8200±200	9500±400	14300±700	28±10	100±20
V	310±60	320±60	340±20		
Cr	260±100	230±20	310±20	100±20	
Mn		30±10		12±6	
Fe	5860±100	6200±200	7700±400	690±10	730±20
Ni	172±4	61±10	96±9	44±4	
Cu	182±3	136±8	95±2	35±10	
Zn	138±4	98±5	64±2	34±5	11.7±0.7
Ga	52±3	60±3	73±6		
Br	24±0.5	24±3	10.3±0.6	10.7±0.6	11±1
Sr	80±1	74±3	41±3	3.6±0.4	10.5±0.5
Zr	240±3	310±10	604±10	84±7	19±1
Mo	5±1				
Cd	12±1	20±5	13±3		22±4
Pb	52±1	35±3	18±1	4.4±0.7	5±2
Th	26±2	28±5	35±5		
Y	19±1	19±2	35±1	40±8	2.6±0.4
Nb	32±2	32±2	45±1	22±4	
Ba	300±50	1410±40	460±20	880±50	
U	6±1	10±2	11±1		
W			9±4		
Bi			6±2		
Se					1.1±0.4
Sc				700±200	
Rb	2.9±0.5		2.4±0.9		
Free Silica (%)	0.7	0.8	0.4	≈100	≈100

*Data presented are the average of two determinations with the standard deviation.

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[1] From Stettler et al., 1981

Table 6. Individual Particle Analyses — Number of Particles Containing Specified Element [1]

	Ottawa Sand	Chromite Sand	North Carolina Olivine	Washington Olivine	Florida Zircon	Australia Zircon	Remasil 48	Remasil 60	Remull	Min-U-Sil
Na	2	3	1	3				1		1
Mg	5	433	960	992				10	3	
Al	6	941	16	22	23	10	1009	1007	1006	7
Si	1023	59	980	1023	992	1020	1018	980	978	1000
P						2	1	1		
S							2			
Cl		6	3		1					
K	4		1	5				1	3	3
Ca	7	18	11	19	4	5	5	31	5	2
Ti	3	20	4	3	14	22	674	835	927	6
Cr	3	987	21	23	3	2	7	2		
Mn	1							1		1
Fe	17	1009	833	990	18	12	176	401	495	19
Ni			3	3	2		4	2		
Cu		3								
Zn		1		1			1	1		
Hf					2	1				
Zr					963	991				
Total Analyzed	1028	1035	1000	1030	1004	1030	1030	1030	1012	1011

Table 7. Individual Particle Analyses — Specific Elemental Combinations Found [1]

	Ottawa Sand	Chromite Sand	North Carolina Olivine	Washington Olivine	Florida Zircon	Australia Zircon	Remasil 48	Remasil 60	Remull	Min-U-Sil
Si (Silica)	1005	4	21	21	10	13	16	19	6	980
Mg-Fe Silicates	1	16	797	952		1				
Mg Silicates		4	131	12		1				
Al Silicates	1			2	13	4	974	881	951	4
Cr Fe-Major	2	980	15	10	3	1	7	2		
Zr Silicates (Pure)		1			952	977				
Zr Silicates (Impure)					10	13				
Al (Oxide)							24	107	51	
Ti (Oxide)			3	1	6	11				5
Fe (Oxide)	7	2	5	3	4		1	1		7
Other Oxide		1	3				4	8	2	
Ca-Mg Silicates	1	2	8			3				
Ca-Mg-Fe Silicates		1	1	12						
Other Silicates	5	2	2		1		1	2		12
Other Al Silicates	5	16	9	10	5	3	2	4	2	3
No X-rays	1	3								
Miscellaneous		3	5	7		3	1	6		
Total	1028	1035	1000	1030	1004	1030	1030	1030	1012	1011

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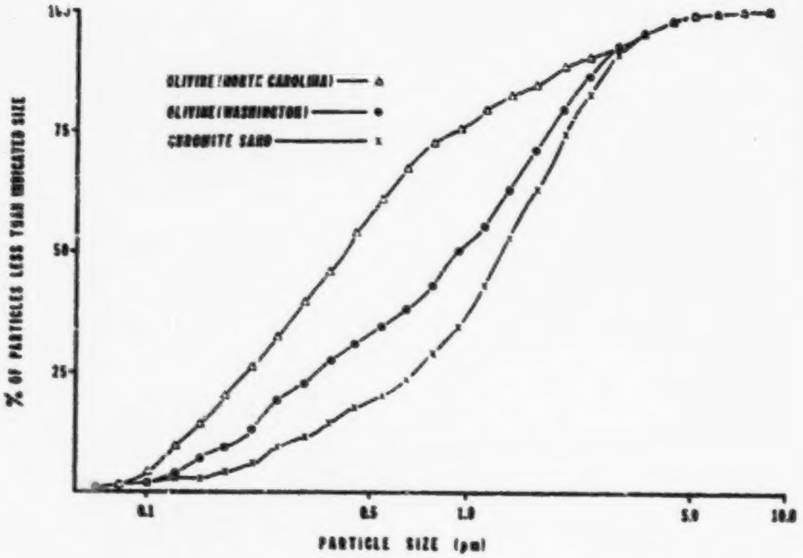
[1] From Stettler et al., 1981

TABLE 8. SUMMARY OF FIBER ANALYSES - TOTAL NUMBER OF FIBERS FOUND

	Respirable Fraction	Original Material
Ottawa Sand	3	0
Chromite Sand	2	38
North Carolina Olivine	11	185
Washington Olivine	27	211
Florida Zircon	2	0
Australian Zircon	2	1
Remasil 48	0	0
Remasil 60	0	0
Remull	0	0
Min-U-Sil	---	1
Blank	0	0
Blank	0	0
Blank	2	1

From Stettler et al., 1981

Fig. 1. Cumulative particle size distributions for the olivine and chromite sand respirable fractions.[1]



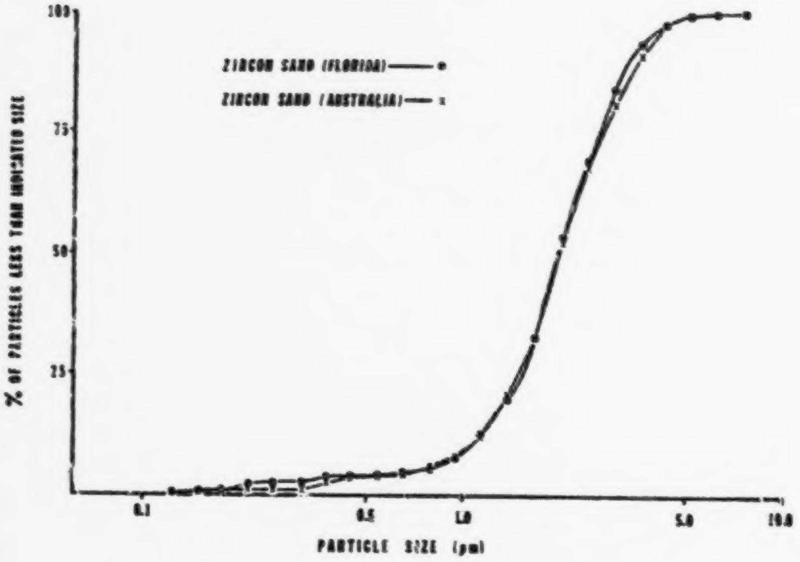


Fig. 2. Cumulative particle size distributions for the zircon sand respirable fractions.[1]

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[1] From Stettler et al., 1981

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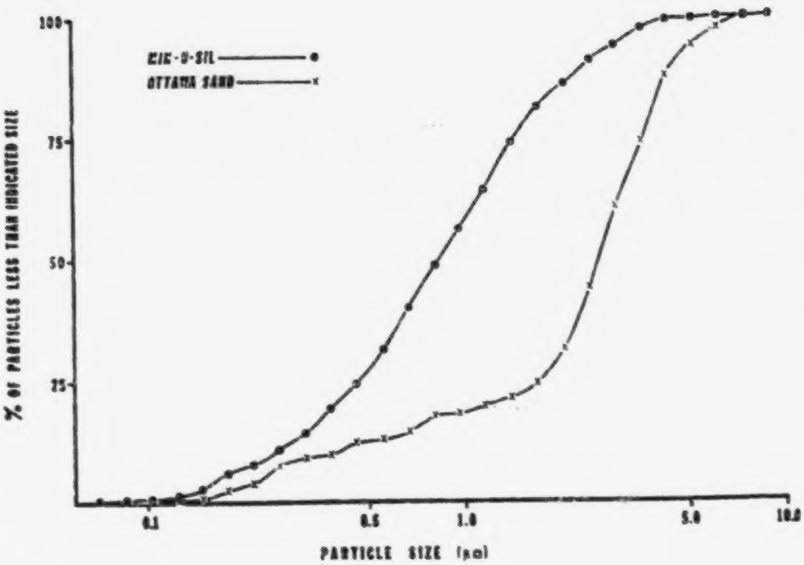

Fig. 3. Cumulative particle size distributions for the Min-U-Sil and Ottawa sand samples. [1]

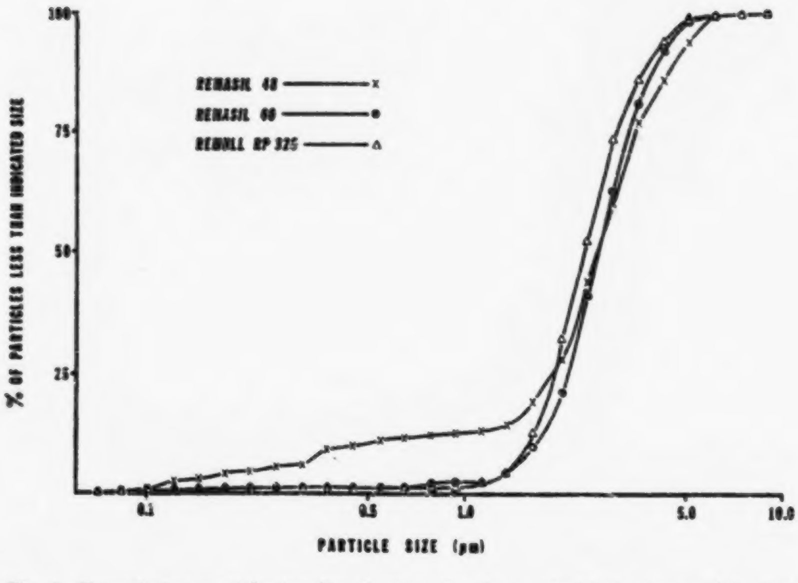

Fig. 4. Cumulative particle size distributions for the respirable fractions of the three aluminum silicate samples. [1]

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[1] From Stettler et al., 1981

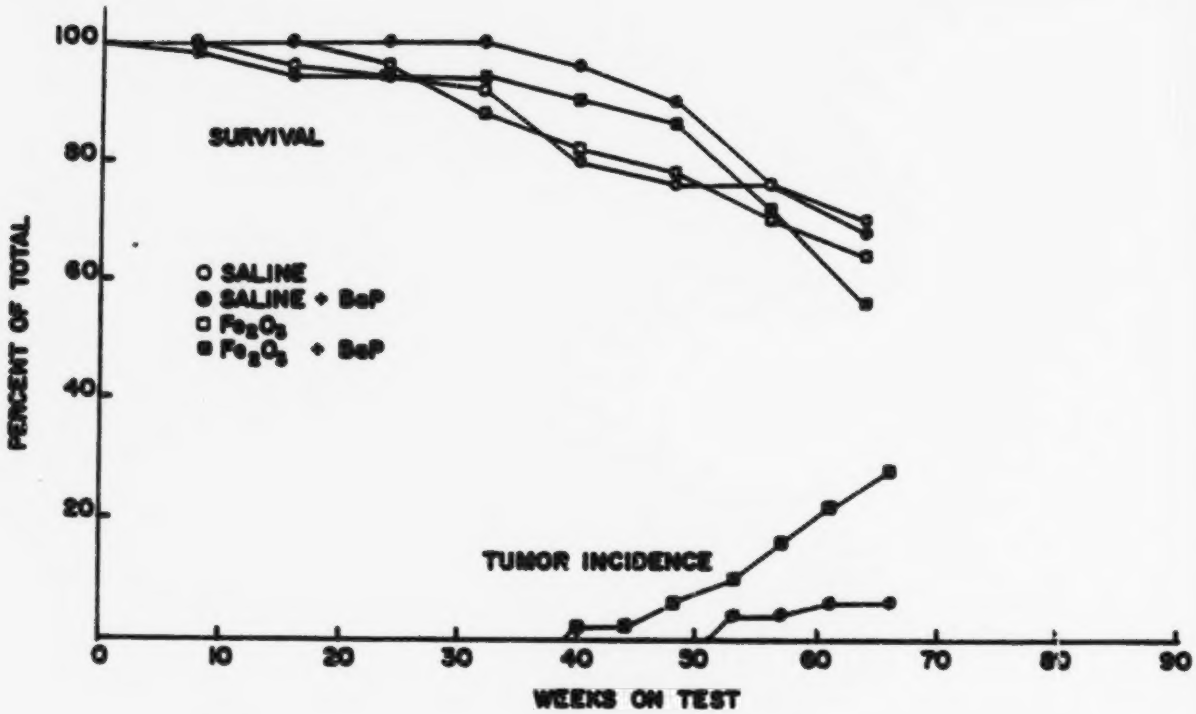

Figure 5. Survival and gross tumor incidence of saline control and ferric oxide groups.

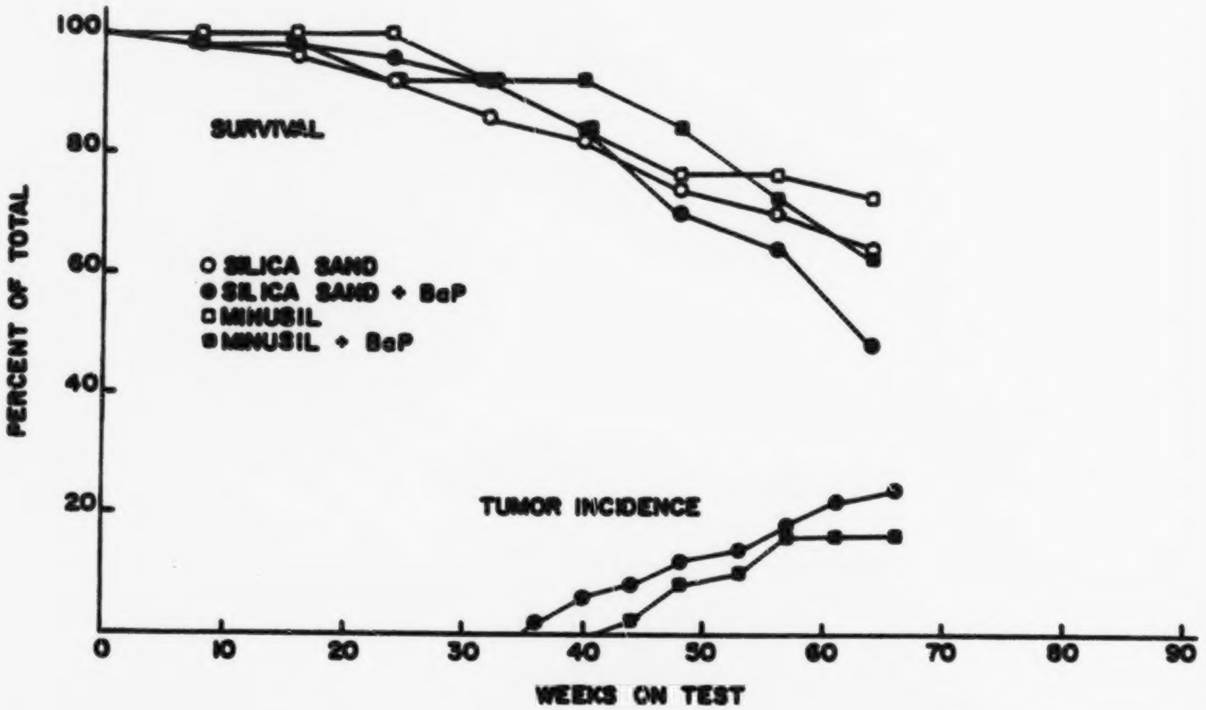

Figure 6. Survival and gross tumor incidence of silica sand and minusil groups.

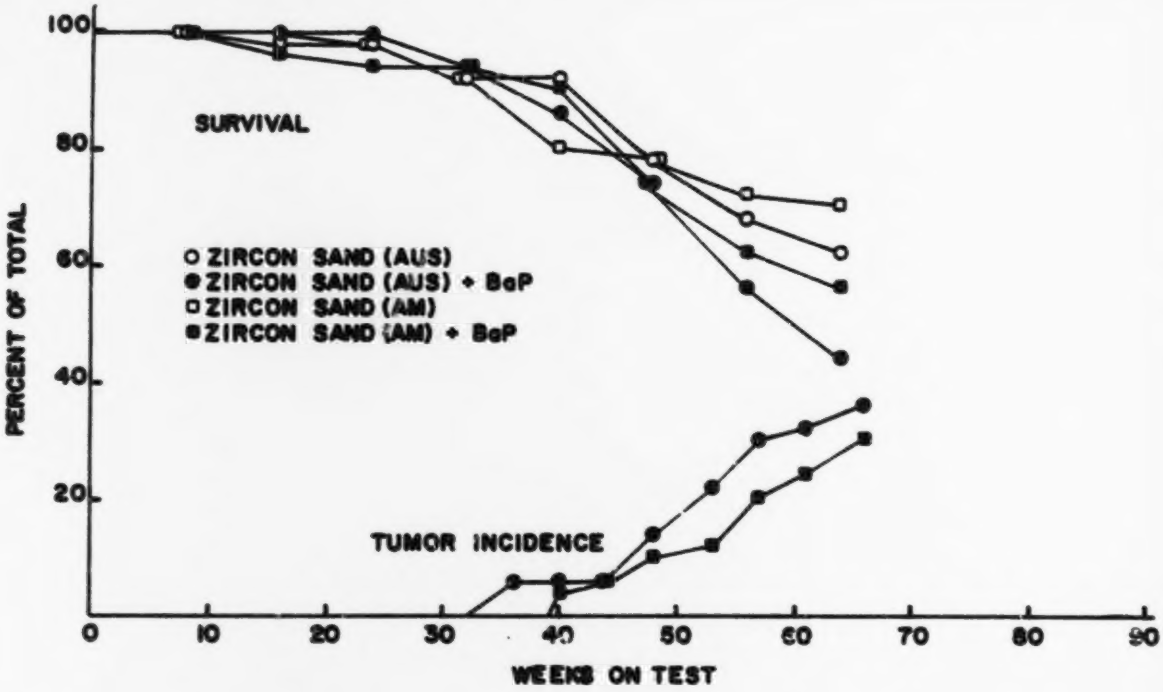

Figure 7. Survival and gross tumor incidence of zircon sand groups from two geographical sources.

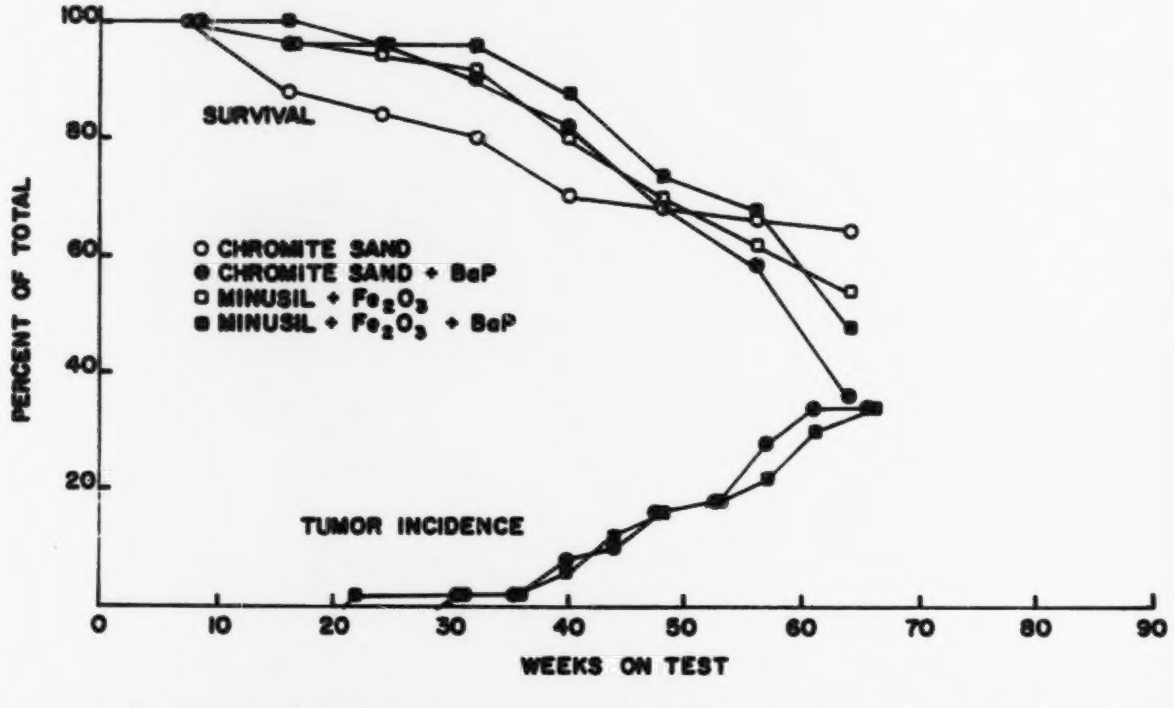

Figure 8. Survival and gross tumor incidence of chromite sand and combination minusil plus ferric oxide groups.

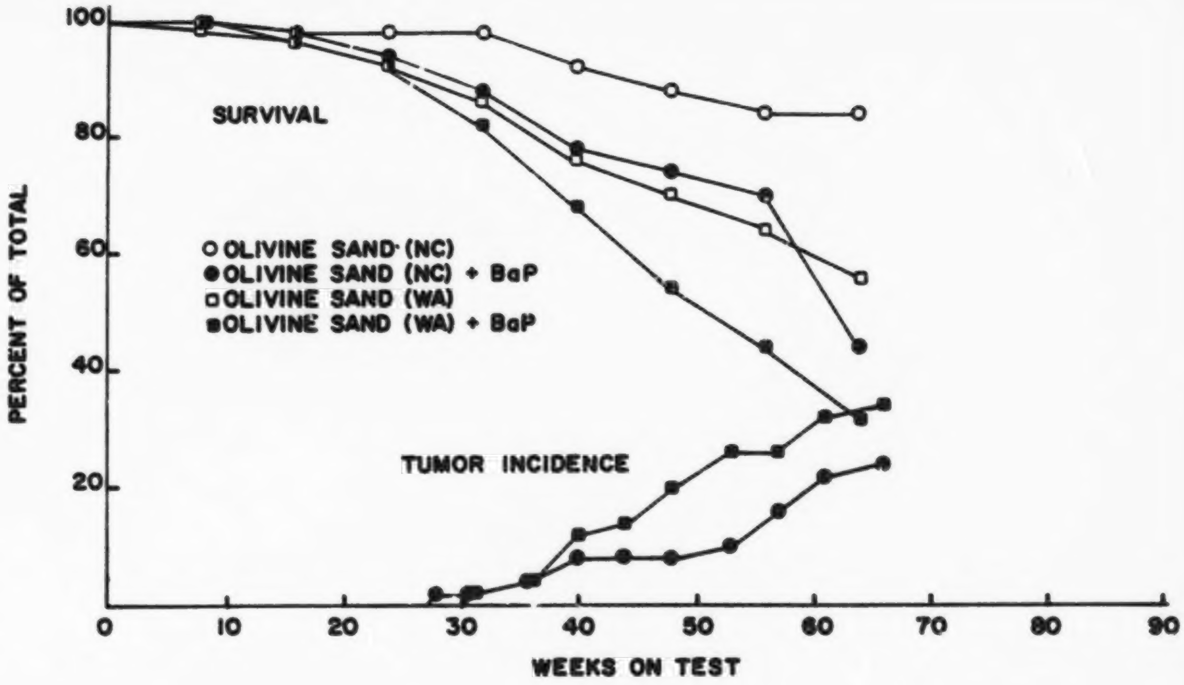

Figure 9. Survival and gross tumor incidence of olivine sand groups from two geographical sources.

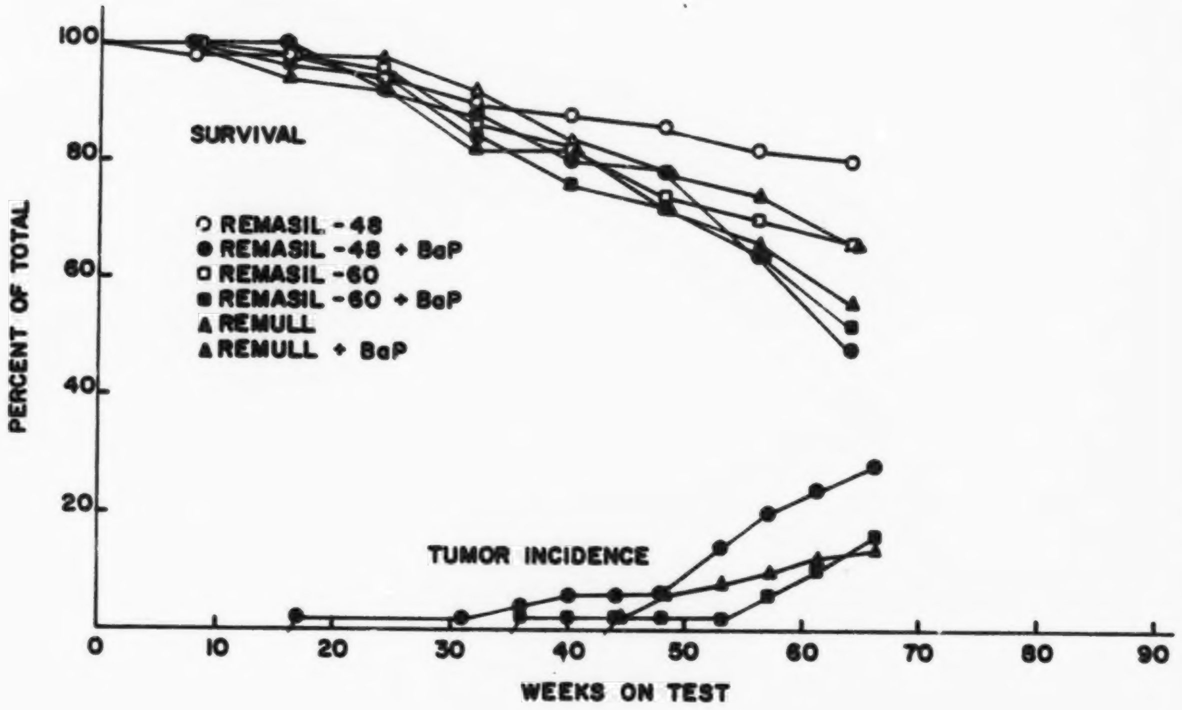

Figure 10. Survival and gross tumor incidence of three aluminum silicate groups.

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PROCEEDINGS OF THE
SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Carcinogenic Potential of Condensed Pyrolysis of Effluents from Iron Foundry Casting Operations

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ABSTRACT

Recent epidemiological studies in the United States and abroad indicate that certain foundry workers, particularly molders and metal pourers, have a significantly higher than normal risk for developing lung cancer. The National Institute for Occupational Safety and Health is currently conducting a study in iron foundries in an effort to determine whether the increased risk can be due to the presence of carcinogens in mold emissions. In this study, emissions from molds composed of four types of binders, including furan, urethane, shell and green sand, are being collected and tested for their ability to induce cancer in hamsters.

Source samples were collected with a wet venturi scrubber from molds shortly after they were poured. The concentration of eight polycyclic aromatic hydrocarbons and of sixteen metals was determined in each sample. The size of the airborne particles was measured at the inlet to the scrubber and also in the collected sample.

Samples are being administered to Syrian golden hamsters by intratracheal instillation. The bioassay is being performed in two sections; the first is a six-week range-finding study and the second is a chronic carcinogenicity assay. The purpose of the range-finding study is to determine the dose to be used in the carcinogenicity bioassay. This dose is the maximum dose that the animals can tolerate without causing clinical disorders, other than cancer, that may shorten their lifespan. The maximally tolerated dose will be repeatedly administered to animals for a period of time sufficient to induce cancer if chemical carcinogens are present in significant quantities.

Abstract

This study is essentially an exploratory investigation which should indicate directions for future research.

Acknowledgement:

This research is supported by a NIOSH/NCI interagency agreement. The work is being conducted under Contract No. 210-78-0033 with Borriston Research Laboratories, Temple Hills, Maryland, 20031.

INTRODUCTION

The purpose of this study is to evaluate the carcinogenic potential of pyrolysis effluents generated during molding and pouring operations at iron foundries. NIOSH and NCI are engaged in this work because of the combined findings of increased risk to lung cancer obtained by epidemiology and the recent finding that known carcinogenic chemicals are generated during various foundry operations. The work being performed can be divided into three distinct phases: collection of foundry mold emissions, analysis of samples collected, and the carcinogen bioassay. The foundry collection and sample analysis phases were completed during 1980. The bioassay is well under way with completion anticipated during 1983.

BACKGROUND

NIOSH has been extensively involved in health effects research for the foundry industry during the past 15 years. The Toxicology Branch, through the efforts of the late Dr. Lester Scheel, completed a study identifying process emissions and occupational health concerns in ferrous foundries(1). A report of an investigation evaluating occupational health hazard control technology has been recently completed(2). Currently, we have studies dealing with such topics as infrared radiation, vibration, particulate characterization, foundry (IH) sampling, cocarcinogenicity of foundry particulates, and the carcinogenic potential of mold effluents. Furthermore, a draft criteria document for the foundry industry has been prepared.

The major health hazards in the foundry industry are respiratory diseases due to high concentrations of dusts and gases. Table I lists the major reports on this subject. The first three investigations were based on census figures and death records. In these studies, it was found that foundry workers, and in particular molders and casters, have higher lung cancer rates than workers in other occupations. In 1938, Turner and Grace reported that foundry workers in Sheffield, England, had the highest lung cancer rates among the 12 occupations examined(3). In 1939, the Registrar General of Great Britain reported that molders and metal pourers ranked fourth among occupations with high lung cancer rates in London and Wales(4). Milham later reported similar findings for foundry workers in the State of Washington(5). In a recent NIOSH study, based on the death records from the International Molders and Allied Workers Union's death benefit program, Egan and coworkers reported that there is a 1½-fold excess of lung cancer cases among foundrymen(6).

The two epidemiological studies which are perhaps the best known were performed in Finland by Koskela et al.(7) and in Canada by Gibson and coworkers(8). Both studies found that molders and cleaning room operators were at a two- to threefold higher than normal risk of developing lung cancer. The Canadian group also found that crane operators are at an elevated risk of lung cancer, but this was not found to be the case in another study of crane operators in steel foundries performed by Lerer et al.(9). Finally, in a recent report of the lung cancer rates in workers in a U.S. foundry, Decoufle and Wood reported that there was a twofold excess of lung cancer in workers with more than five years' exposure in the foundries(10).

We hypothesize that an elevated risk of lung cancer exists in certain areas of foundries as a result of exposure to thermal decomposition products evolving from pyrolyzed mold sand binders. Furthermore, the complex emissions vary with different binder composition, resulting in different carcinogenic potential.

APPROACH AND RESULTS

I. Foundry Sampling Phase.

As a result of the known differences in mold emissions emanating from different organic sand binders, we have selected the four most common types of molds to sample. The four types include (1) common green sand composed of sand, clay, cereal, seacoal; (2) shell type molds with phenol-formaldehyde; (3) urethane molds using phenolic isocyanate; and (4) furan molds of urea-formaldehyde-furfuryl alcohol.

Mold emissions were collected in foundries which poured gray iron into molds under conditions in which emissions from cooling molds could be collected in the absence of significant cross-contamination by airborne emissions from other processes, such as melting or shakeout. In an actual foundry operation, this is difficult to accomplish. To help achieve this, samples were collected in isolated areas, such as cooling tunnels, wherever possible. Thus, foundries were sought in which cross-contamination was minimized either by appropriate local ventilation of nearby processes or by the absence of other fume- or dust-emitting processes in the vicinity of the mold cooling area.

Another important criterion in the selection of foundries was that there were no emissions present from sand binders other than the particular binder being studied. Therefore, it was essential to collect samples from molds in which cores, when present, were composed of the same chemical binders as the molds. Also, reclaimed sand could only be taken from pouring lines using the same chemical binders as the molds being sampled in order to avoid contamination from other resins remaining associated with the recycled sand.

For use in this study, a sampling method was sought which would enable the efficient collection of those components of mold emissions that would naturally be retained by the respiratory tract during exposure by inhalation. The large quantities (approximately 50g) of concentrated materials (50 to 100 mg/ml water) required for the animal carcinogenicity tests necessitated the use of a high volume sampler from which the samples could be retrieved in a concentrated form. Since the samples were administered to animals in an aqueous medium, a sampler which could trap mold emissions in water was desired.

Figure 1 shows a schematic drawing of the scrubber. Air enters a cyclone precleaner at the rate of 100 cfm. This cyclone eliminates particles with diameters in the range of 10 microns and higher. The rejected particles gather in the bottom of the cyclone precleaner. The air discharged from the cyclone passes into the top of the venturi scrubber where it impinges on a stream of water. When this occurs, the water is dispersed into a mist. Particles greater than 0.2 microns are captured by the water droplets. The airstream takes a downward spiraling path through the scrubber and is then directed into the entrainment separator. Here, by cyclonic action, the particle-laden mist coalesces into water droplets which collect in a sump at the bottom of the separator. Water is removed from the sump and is recirculated to the venturi scrubber, where it is reused for further sample collection. In this way, the concentration of collected materials in the water increases with time.

II. Analytical Characterization Phase.

The samples collected from the cooling molds consist of a water soluble fraction and a particulate fraction that was relatively insoluble in water. From our work, as well as from data published by other authors, it is known that emissions from cooling molds contain a wide range of chemical and mineral components. Among the determinations performed were measurements of the concentrations of 8 polycyclic aromatic hydrocarbons (PAH) in both the water-soluble and insoluble fractions of the samples. The levels of some of the phenols in the aqueous phase and the concentrations of metals in the whole sample were also measured.

The elemental analysis of a typical foundry sample is shown in Table 2. Sixteen elements were determined by atomic absorption. A separate determination of silica gave the value of 3.3%. A comparison between the metal analyses of the emissions from the four types of mold binders indicated that the mold binder had no apparent effect on the metal concentrations of the mold effluents. The concentrations of certain metals were consistently lower than what might be expected in the emissions on the basis of the metallurgical content of the poured iron. These data show that the metals that are most readily released into the mold emissions are those with the highest vapor pressure at the pouring temperature.

Hundreds of PAH's have been identified evolving from pyrolyzed organic material similar to the binder chemicals used in the four foundry mold type being studied. Eight PAH's were selected for analysis to characterize complex effluent mixtures by such features as number of arene rings, nitrogen content, and heterocyclic character.

The concentrations of the PAH's in the cyclohexane extracts of particulates from all four foundry samples are shown in Table 3. The data are presented in terms of μg PAH per gram particulate. Since two of the GC/MS peaks contained two PAH each, the analyses were also performed by HPLC (high pressure liquid chromatography). Although this technique tends to be more subject to interferences for PAH than is GC/MS, it can differentiate between certain combinations of compounds, such as benzo(a)anthracene and chrysene, which were not distinguished by GC/MS. Table 3 shows the combined concentrations, as determined by GC/MS, of benzo(a)anthracene and chrysene. Using HPLC, it was found that chrysene represented a considerably greater portion of the total benzo(a)anthracene/chrysene GC/MS peak than did benzo(a)anthracene in three of the four foundry samples. Of these two compounds, only chrysene was detected in the furan sample and there was approximately four times as much chrysene as benzo(a)anthracene in the urethane and shell samples. The opposite situation occurred in the green sand sample which had about five times as much benzo(a)anthracene as chrysene. There were no detectable quantities of either acridine, carbazole, or dibenz(a,h)anthracene in any of the samples. Naphthalene was found in only the urethane sample and phenanthrene was present in all four samples. Measurable quantities of Benzo(a)pyrene were only present in the shell and green sand samples.

The last series of compounds that were determined were the phenols. They were studied because of their ability to promote tumors in some animal bioassay systems. These are all highly soluble in water and hence only the aqueous phase was examined. Of these compounds, phenol itself was the most prevalent and was found in all four foundry samples. 4-Nitrophenol, the second most prevalent phenol, was present in all but the green sand sample. 2-Nitrophenol was found in the shell sample only and 2,4-dimethylphenol was found in the shell and urethane samples (Table 4).

III. The Carcinogen Bioassay Phase.

The bioassay is being performed in two parts. First, acute range-finding studies were completed to determine maximum tolerated doses (MTD) and second, the life-long studies were initiated and are currently underway. This approach is similar to that described by Saffiotti _et_ _al_.(11)

Golden Syrian hamsters (7 weeks of age) of both sexes were purchased from Charles River Breeding Laboratories (Wilmington, Massachusetts) following serological screening. Prior to placement on study, all animals were quarantined for a period of two weeks. All animals were individually housed in solid polycarbonate cages with sterile bedding and suspended from stainless steel racks equipped with nonwoven filter bonnets. Animals were kept under optimum hygienic conditions in environmentally controlled rooms.

Animals were anesthetized with an intraperitoneal injection of 0.5 ml of a 1% solution of Brevital® Sodium. The test articles were administered intratracheally through a 0.25 ml tuberculin syringe fitted with a blunt, bent 19 gauge needle. A dosing volume of 0.2 ml was used for all groups. The needle was inserted gently under the epiglottis into a tracheal lumen, the suspension gently injected, and the needle withdrawn. Inspection of the pharynx was continued for a short period and the animals were kept on a restraining board for a minute or two to assure that no suspension was regurgitated.

On the basis of the data from the acute studies (observation of animals during instillation, mortality, body weight, and histopathology), two dose levels, MTD and 1/2 MTD, were selected for administration in the chronic bioassay.

Eighteen weekly intratracheal doses of 10.0 mg and 5.0 mg for the Green Sand, Shell, and Furan samples, and of 5.0 mg and 2.5 mg for the Urethane sample were used. All samples were titrated to a pH of 7.0 prior to instillation with 0.1 N NaOH. A positive and negative control treatment was used. The positive control group received a 0.2 ml of a mixture of 3 mg benzo(a)pyrene and 3 mg of ferric oxide. The negative control group received 0.2 ml of distilled water. All treatment groups for the chronic bioassay consisted of 50 male and 50 female hamsters. At the present time, the chronic IT dosing has been completed. A final report will be available during 1983.

DISCUSSIONS AND CONCLUSIONS

The primary objective of this project is to evaluate the potential carcinogenicity of complex mixtures in foundry mold effluents. In pursuit of this objective a number of accomplishments have occurred. A custom-designed high volume wet venturi scrubber was developed which will provide technology for future investigations requiring large quantities of complex airborne emissions for bioassays. Additionally, analytical characterization of emissions from cooling molds of the four common types has been completed. This will provide industrial hygienists, toxicologists, and control technologists with guides for future research.

The acute range-finding bioassay has demonstrated similar acute toxicities for the green sand, furan, and shell molds. The urethane samples were far more toxic acutely, which resulted in the determination of a maximum tolerated dose of one-half that for other mold samples.

In conclusion, many factors contribute to the chronic toxicity or carcinogenicity of airborne pollutants. The actual concentrations of substances that can act as chemical carcinogens, the presence of other compounds which can act as tumor promoters or cocarcinogens, and the characteristics of the particulates with which they are associated all play a crucial role in determining the health hazards associated with inhaled pollutants. Long-term animal tests are currently accepted as the best available method for assessing the combined toxicity of the many components of mold emissions, as well as those from other combustion processes.

Table 1. REPORTS OF EXCESS LUNG CANCER AMONG IRON FOUNDRY WORKERS

Report	Finding
Registrar General, Great Britain, 1939	Molders and pourers ranked 4th among occupations with high lung cancer rates
Turner and Grace, 1938	Foundry workers had highest lung cancer rates among 12 occupations in Sheffield, England
Milham, 1976	Molders had excess cases of lung cancer in Washington State
Egan *et al.*, 1979	1.5-fold excess lung cancer among participants in Union Death Benefits Program
Koskela *et al.*, 1976	>2-fold excess among molders with >5 years' exposure
Gibson *et al.*, 1977	>2-fold excess among crane operators, molders, and finishers
Decoufle and Wood, 1979	>2-fold excess among all workers with >5 years' exposure

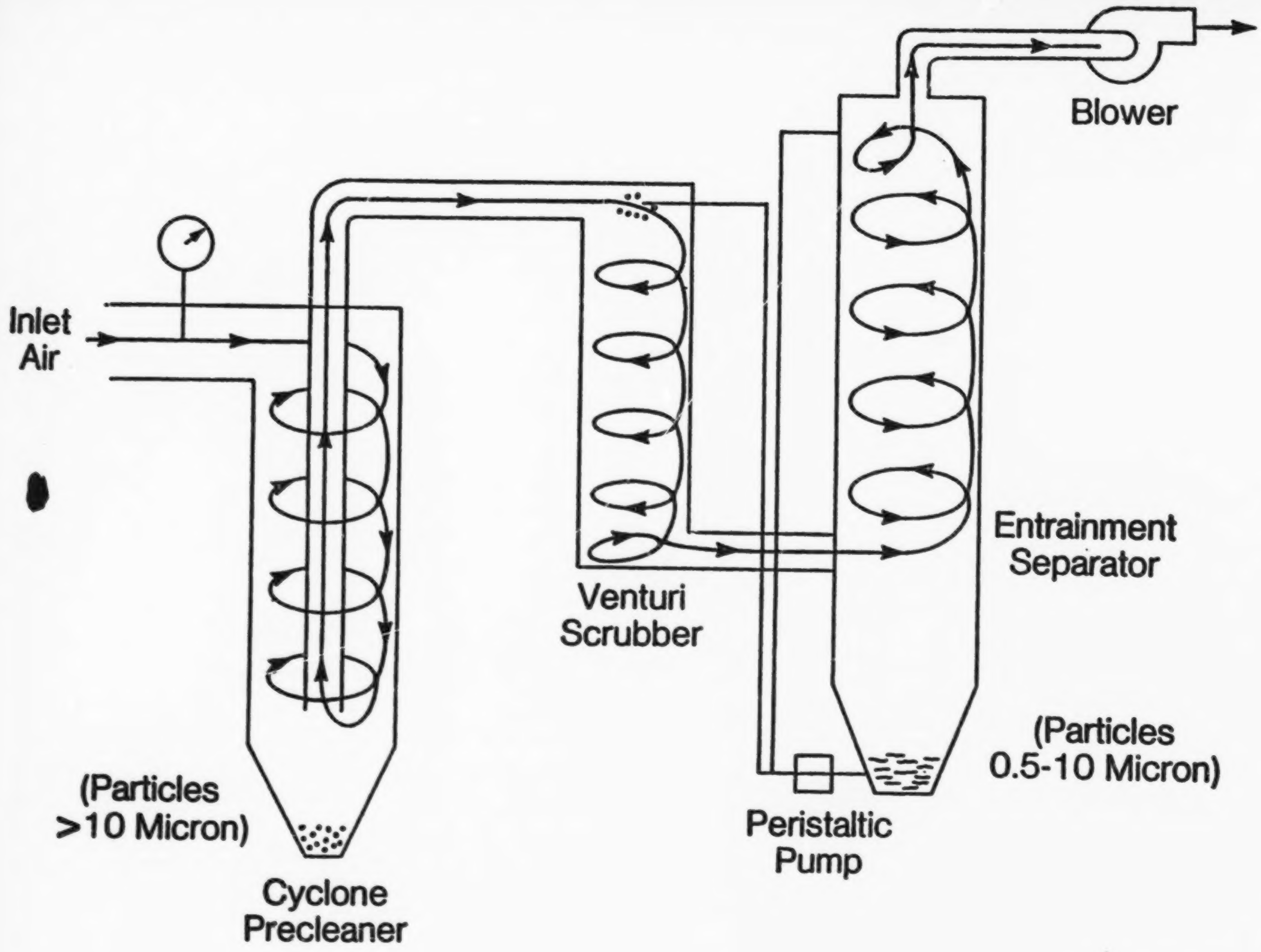
Blower
Inlet Air
Entrainment Separator
Venturi Scrubber
(Particles 0.5-10 Micron)
(Particles >10 Micron)
Peristaltic Pump
Cyclone Precleaner

FIGURE I

Table 2. Elemental Analysis of a Typical Sample of Mold Emissions

Metal	Concentration (mg/gm sample)
Aluminum	0.31
Arsenic	0.013
Beryllium	0.003
Cadmium	0.022
Chromium	<0.0055
Cobalt	0.055
Copper	3.9
Iron	8.08
Lead	0.36
Magnesium	0.023
Manganese	1.71
Mercury	<0.0001
Nickel	0.03
Silicon	1.6
Tin	0.114
Zinc	1.6

Table 3. CONCENTRATIONS OF PAH AND RELATED COMPOUNDS IN CYCLOHEXANE EXTRACTS OF WATER-INSOLUBLE PARTICULATE (μg/g PARTICULATE)

	COMPOUND	FURAN	URETHANE	SHELL	GREEN SAND
0	ACRIDINE	< 0.6	< 0.6	< 0.6	< 0.6
0	NAPHTHALENE	< 0.6	12	< 0.6	< 0.6
0	CARBAZOLE	< 0.6	< 0.6	< 0.6	< 0.6
0	PHENANTHRENE	31	230	1500	7200
+	BENZO(a)ANTHRACENE }	< 0.6	5.4	350	1100
+,0	CHRYSENE }				
++	BENZO(a)PYRENE	< 0.6	< 0.6	270	230
++	DIBENZO(a,h) ANTHRACENE	< 0.6	< 0.6	< 0.6	< 0.6

---

(1) 0.6 lower detectable level

(2) 0 noncarcinogenic
+ weakly carcinogenic
++ strongly carcinogenic

Table 4. CONCENTRATIONS OF PHENOLS IN AQUEOUS PHASE (μg/g SAMPLE)

COMPOUND	FURAN	URETHANE	SHELL	GREEN SAND
PHENOL	2000	50000	1600	1000
PENTACHLOROPHENOL	3	< 2*	< 2	< 2
4-NITROPHENOL	48	420	1800	< 2
2-NITROPHENOL	< 2	< 2	2300	< 2
2,4-DIMETHYLPHENOL	< 2	21	60	< 2

*Lower level of detection

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PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Determining the Role of Pulmonary Fibrosis in the Etiology of Lung Cancer

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ABSTRACT

Epidemiological evidence reveals an increased lung cancer risk for workers in various silicious trades. Many of these cancers appear to originate from areas of fibrosis. Animal studies have demonstrated that concomitant exposure to various particulates and a carcinogen is more effective than either one alone in the induction of pulmonary carcinoma. Based upon these observations, numerous investigators have questioned the interactive roles of agents which cause pulmonary cellular proliferative changes and pulmonary cancer, for example, in such occupational groups as hematite miners, and foundry, asbestos, and chromate workers.

This report gives the current status of the first phase of a joint NIOSH-NCI effort to develop an experimental model which addresses the hypothesis that pulmonary fibrosis presents a cocarcinogenic effect in the etiology of pulmonary carcinoma. The second phase will be conducted in a future study using the animal model exposed simultaneously to the fibrogenic agent and a carcinogen, such as benzo(a)pyrene.

As the first phase in testing the hypothesis, this study was designed to investigate the type and extent of pulmonary fibrotic response induced in male Syrian Golden hamsters given multiple intratracheal instillations of crystalline quartz, fibrous glass, hydrated alumina, or a 1:1 mixture of ferric oxide and crystalline quartz. Doses of each material were chosen, based upon data in the literature, to determine the concentration of each material which induces an intense pulmonary fibrosis with multiple instillations, but does not alter the normal lifespan of the animal.

Four hundred fifty male hamsters were divided equally into 18 treatment groups, i.e., 4 materials X 4 doses, plus saline and cage controls. Test materials, in 0.5 ml sterile saline, were administered once weekly for 15 weeks. The biological endpoints include data derived from biweekly body weighings, daily clinical sign observations, and gross and microscopic pathology of trachea, lung, and associated lymph nodes.

Interim results will be reported and show that at the end of week 82, there was an overall rate of 58% mortality. The two highest dose groups of quartz and quartz plus ferric oxide have been terminated because spontaneous mortality in these groups reached 80%. Results also reveal that amyloidosis has been a cause of death in many of the hamsters which died or were sacrificed.

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INTRODUCTION

Numerous investigators have pondered the interactive roles of agents which cause pulmonary connective tissue proliferation and pulmonary cancer. The association between pulmonary scars and malignancy was made by Raeburn (1951) and Raeburn and Spencer (1953). Faulds and Stewart (1956) suggested a causative association of iron ore mining and lung cancers; these cancers appeared to originate from areas of fibrosis in the peripheral lung. Animal studies demonstrate that coupling a carcinogen to asbestos fibers or iron oxide increases its ability to induce pulmonary carcinoma (Kennedy and Little, 1974; Wilcox, Marcussen and Furst, 1974; Schreiber et al., 1974). Boyd and coworkers (1970) noted that lung cancer mortality in hematite miners working underground was approximately 70 percent higher than in the local non-mining population. Epidemiological evidence exists for an increased lung cancer risk in workers in various silicious trades, e.g., molders and foundrymen (McLaughlin and Harding, 1956), metal grinders (Kennaway and Kennaway, 1947), and chromate workers (Bidstrup and Case, 1956). All these observations suggest that exposure to iron or silica, or a combination of both with other materials, may predispose workers to pulmonary cancer.

Attempts have been made to produce experimentally in animals types of lung cancer commonly seen in man. Both inhalation and intratracheal instillation of substances have been tried in rats and hamsters. Although good results have been obtained with rats, Syrian Golden hamsters are preferred for use in intratracheal instillation studies because of their lower incidence of spontaneous lung disease (Homberger, 1969).

The purpose of this study is to determine the type and extent of fibrotic response induced by crystalline quartz, fibrous glass, hydrated alumina, and a 1:1 mixture (w/w) of ferric oxide and crystalline quartz which have been reported to induce pulmonary fibrosis and which present a potential occupational hazard. The specific objective of the study is to induce varying degrees of pulmonary fibrosis with each material by the end of the natural lifespan of exposed male Syrian Golden hamsters. This information will be used to determine a dose of one or more of these materials which induces significant pulmonary fibrosis but does not alter the normal lifespan of the hamster. Further studies will assess the role of pulmonary fibrosis in pulmonary carcinogenesis in this animal model by simultaneously exposing hamsters to this dose of material and a known carcinogen, such as benzo(a) pyrene.

This is an interim report containing progress and results from the period September 27, 1979, the date of the contract initiation, through June 23, 1981. The final report will be available in May 1982.

MATERIALS AND METHODS

The hydrated alumina, ferric oxide, and crystalline quartz samples were physically characterized by NIOSH as to median, average, mass median, and aerodynamic diameters. The fibrous glass sample was characterized by median diameter, median fiber length, number of fibers longer than 10 micrometers, and number of fibers wider than 3.2 micrometers. These dimensions were to verify the specification that the fiberglass sample was to be greater than 10 micrometers in length and less than 3.5 micrometers in diameter.

Samples of the particles were prepared from the bulk powders by dispersing a small quantity of the powder in a 0.05% solution of Aerosol OT in de-ionized water. The solutions were placed in an ultrasonic bath for ten minutes and then stirred magnetically for one-half hour. Aliquots of these suspensions were then filtered through a 0.1 micrometer pore size Nucleopore filter. The filters were attached to carbon planchets with colloidal graphite and examined directly with a scanning electron microscope (JOEL, Model JXA-50A), equipped with an energy dispersive X-ray spectrometer system (EG & E Ortec Model EEDS II) and an image analysis system (LeMont Scientific, Model B-10) using the back-scattered electron image. A minimum of 1,000 particles of each samples was sized.

Table 1 gives the results of the physical characterization of the four materials instilled intratracheally. The ferric oxide sample was highly aggregated; the ultimate particle size appeared to be 0.02 μm. The large mass median and mass median aerodynamic diameter for the hydrated alumina sample indicate the presence of a few very large particles in the sample, which on a weight basis were a considerable part of the sample. The fibrous glass sample had approximately ten percent of the fibers with a length greater than 10 μm. On a weight basis, this was 80 to 90 percent of the sample; however, on a number basis, most of the fibrous glass sample consisted of short (< 10 μm) fibers.

Table 1. Physical Sizing of Materials

Compound	Particles	Diameters (micrometers)			
		Median	Average	Mass Median	Mass Median Aerodynamic
Quartz (6 samples)	1048 ± 70	0.84 ± 0.07	1.06 ± 0.07	3.14 ± 0.24	5.13 ± 0.40
Ferric Oxide (1 sample)	1754	0.27	0.29	0.60	1.37
Alumina (1 sample)	1002	0.50	0.81	6.31	9.81
		Median Diameter	Median Length	No. (lengths) > 10 μm	No. (width) > 3.2 μm
Fibrous glass	1017	1.88	2.97	102	30

Individual doses per weekly instillation were as follows: (1) quartz--0.03, 0.33, 3.3, and 6.0 mg; (2) quartz plus ferric oxide--0.03, 0.33, 3.3, and 6.0 mg each; (3) fibrous glass--0.05, 0.5, 1.0, and 10.0 mg; and (4) alumina--0.2, 2.0, 5.0, and 20.0 mg.

Five hundred (500) male Syrian Golden hamsters were purchased in one lot at six weeks of age from Charles River Laboratories (Lakeview) Wilmington, Massachusetts. Ten of them were necropsied upon arrival. Lung and nasopharynx were cultured for bacterial pathogens; histopathology was performed on lung, trachea, liver, ileum, and any gross lesions; and serum samples were screened for viral antibodies by Microbiological Associates in Bethesda, Maryland.

Hamsters were individually identified individually by ear notching and housed individually in a wire-bottom 18-gauge, 3/8-inch mesh, stainless steel cage. A standard laboratory diet was fed. Fresh feed and water were available ad libitum. Temperature in the animal rooms was controlled to 23 ± 2°C; relative humidity was controlled at 50 ± 15 percent.

Four hundred fifty (450) hamsters, approximately eleven weeks of age, were divided into 18 groups of 25 animals each (four materials times four doses plus one saline control group and one cage control group). Test materials (crystalline quartz, fibrous glass, hydrated alumina or a 1:1 mixture (w/w) of ferric oxide and crystalline quartz) were administered in a 0.5 ml volume of sterile physiological saline by intratracheal instillation weekly for 15 weeks. Those hamsters that died during the first two instillations were replaced; the replacements were instilled a total of 15 times. Hamsters dying after the second instillation were not replaced. Due to mortality resulting from anesthesia or improper instillation technique, i.e., if there was evidence of perforation, rupture, or massive hemorrhage of the larynx, trachea, or lungs, the number of effective animals was determined for each treatment group. The effective number of hamsters per treatment was equal to the original group size of 25 minus the number of technique or anesthesia related deaths plus the number of animals replaced per group. The resultant size of each group varied from 23 to 26 hamsters. The group size of 26 resulted from the erroneous assumption that one animal died due to the instillation technique; necropsy revealed that this animal died from other causes. The series of 15 weekly intratracheal instillations began in December 1979 and concluded in March 1980.

For the repeated intratracheal instillations, each hamster was anesthesized with sodium brevital (methohexital sodium, Eli Lilly and Company, Indianapolis, Indiana). A dose of 0.42 ml of a 1% solution of sodium brevital per 100 gram body weight was used to provide a level of anesthesia sufficient to allow intratracheal instillation without danger of death from respiratory distress. An intratracheal speculum was inserted through the larynx into the upper section of the trachea, and the test material instilled into the lungs via a catheter inserted into the speculum.

Immediately before each instillation procedure, the appropriate amount of each particulate material was removed from its container, weighed and placed into a sterile flask containing the sterile saline solution. To insure adequate dispersal of the material in the saline solution, the suspension was sonicated for 15 minutes at 200 watts. During the intratracheal instillation procedure, the suspension being instilled was kept homogenous by stirring on a magnetic stirrer.

Hamsters were observed daily by animal care personnel for clinical signs with special attention given to respiratory signs such as labored breathing, coughing, or nasal discharge. Each animal was weighed at the time of ear notching, weekly during the fifteen instillations, biweekly throughout the postexposure period, and immediately before necropsy. The hamsters will be held until 24.5 months of age, or until moribund, at which time they are killed and necropsied. When a group of animals is reduced to 20 percent of the original number, all remaining hamsters in that group are killed.

Hamsters were killed by intraperitoneal injection of a lethal dose of sodium pentobarbital. The thoracic cavity was opened and the trachea and lungs examined in situ. A gross examination of the remaining viscera, mammary glands, external genitalia and musculoskeletal system followed. Lungs were inflated with 10 percent neutral buffered formalin to a pressure of 30 cm of water. One section through the middle of each lobe of the lung, the proximal trachea and tracheal bifurcation, the tracheobronchial lymph nodes, and the mediastinal lymph node was trimmed and processed for microscopic examination. Sections from the heart, liver, stomach, kidney, spleen, and any abnormal tissue or mass observed at necropsy were stored in formalin but not routinely processed for microscopic examination. Processed tissue samples were stained with hematoxylin and eosin. Additional sections and/or special stains were prepared on selected tissues as required for evaluation of lesions, on an individual animal basis. These included connective tissue stains for quantitation of fibrosis, if required.

All histopathologic examination was performed by a board certified veterinary pathologist. This histopathologic evaluation included "blind" comparison of selected tissues of hamsters from various groups to eliminate potential bias. This was accomplished by first examining tissues from animals, with the groups identified to determine the types of lesions produced, then re-evaluating pertinent lesions and comparable tissues from unaffected animals by randomizing the slides and re-evaluating these tissues without knowing the group from which each animal originated. Such technique was especially effective in evaluating somewhat subjective lesions, such as degree of severity of pulmonary fibrosis, inflammation, or epithelial hyperplasia.

Severity of pertinent lesions were graded according to a numerical system denoting whether the response was minimal, slight, moderate, moderately severe/high or severe/high. This procedure permitted an accurate comparison of the degree of pulmonary fibrosis, inflammation, and epithelial hyperplasia induced by the various particulate materials.

A record was maintained on each individual hamster specifying the date and cause of death, type and location of fibrotic and other lesions in the respiratory tract, and lung weight at necropsy.

RESULTS

Electron microscopic sizing of two of the materials subsequently instilled in the lungs of hamsters demonstrated the presence of large particles which were actually aggregates of small particles. These large particles found in the ferric oxide and hydrated alumina samples were not dissociated by the normal low level ultrasonication used to prepare the sample for microscopic examination. The latter is comparable to that used by the contractor to prepare the suspension for intratracheal instillation. Thus, the size data obtained accurately reflects the size of the particles the hamsters received.

The number of animals remaining per treatment group and their mean body weight in grams at weeks 55 and 81 following initiation of intratracheal administration are presented in Tables 2 and 3. Data from Table 2 demonstrate the increased mortality in hamsters receiving the two higher doses of quartz and quartz plus ferric oxide; mortalities were similar for all dose levels of alumina and fibrous glass to that of the saline controls. Table 3 shows the body weight and mortality data at week 81. Decreased body weight is apparent in the highest doses of all surviving groups. Increased mortality is very evident at the two higher doses of quartz and quartz plus ferric oxide; zero values in Table 3 denote that survival in these groups had reached 20 percent and the survivors were killed. No evidence of increased mortality or decreased body weight is present in the fibrous glass group. The hydrated alumina produced the unanticipated result of increased mortality at the lowest doses.

Table 4 presents the survival data for each treatment from week zero (prior to intratracheal instillation) through week 80 (weeks following initiation of IT instillation). Survival rates for all treatments with the exception of the two highest doses of quartz and quartz plus ferric oxide and the two lowest doses of hydrated alumina are comparable to the saline and cage controls. The increased mortality observed in the lower doses of hydrated alumina occurred late in the study, i.e., at weeks 50 to 70.

Selected slides will be shown demonstrating: (1) generalized edema present in two hamsters exposed to 6.0 mg each of quartz plus ferric oxide and one hamster exposed to 6.0 mg of quartz; (2) excellent distribution of instilled material throughout the lung parenchyma; and (3) lung lesions in response to instilled quartz.

DISCUSSION

Development of the experimental technique for instilling a carcinogen such as benzo(a)pyrene (BaP) bound to ferric oxide dust, directly into the trachea (Saffiotti, 1968) represented a significant advance in respiratory carcinogenesis research. A considerable amount of research has been performed in attempting to determine the role of the carrier dust in the induction of respiratory tract tumors with Saffiotti's method (Creasia and Nettesheim, 1974; Stenback, 1974; Post, et al., 1973; Harris, et al., 1971). However, the exact role of the carrier dust in the induction of neoplasia remains unclear.

One of the proposed mechanisms by which ferric oxide exerts its cocarcinogenic effect is stimulation of cellular proliferation (Creasia and Nettesheim, 1974). It seems logical that dusts such as silica, which stimulate intense granulomatous inflammation, fibrosis, and epithelial hyperplasia, may possess greater potential cocarcinogenic properties than ferric oxide. Experimental studies are lacking in which intense pulmonary fibrosis is induced by particulate materials coincidentally with exposure to a potent pulmonary carcinogen such as BaP. Such studies would help to determine the role of stimulation of cellular proliferation by particulate material in the pathogenesis of respiratory tract cancer.

The first step in determining the role of pulmonary fibrosis in the pathogenesis of pulmonary neoplasia is the development of an animal model in which severe pulmonary fibrosis is induced by particulates, but lifespan is not affected. This model must also be susceptible to pulmonary carcinogenesis in response to carcinogens such as BaP. If the particulate materials used to induce pulmonary fibrosis in the model are minerals which are present in the environment in potentially high concentrations, and under certain environmental or occupational situations may be present in combination with potentially carcinogenic aromatic hydrocarbons or other carcinogens, one can assess the role of these particulate materials as potential pulmonary cocarcinogens, while developing an excellent animal model to study the pathogenesis of pulmonary neoplasia.

The terminal sacrifice is scheduled for October 1981. Results to date are satisfactory in terms of meeting the objective of determining a dose of one or more materials which will induce marked pulmonary fibrosis but not compromise the lifespan of the hamster. Once the problem of a proper anesthetic dose of brevital® sodium was resolved, the intratracheal instillations progressed well with only a few deaths attributable to dosing. Microscopic examination of lung tissue revealed an excellent distribution of the instilled material throughout the lungs. The ultimate success of the study is dependent on the pulmonary pathology in evidence at twenty-four and one-half months of age in the hamster for each treatment group surviving to that time.

Amyloidosis, a disease frequently observed in adult hamsters, has been the cause of or contributor to the death of many of the hamsters which died or were killed to date. The experimental regimen appears to have exacerbated this genetically predisposed disease in the hamsters in which the materials were instilled, i.e., cage and saline control groups have had a lower incidence of amyloidosis as compared to hamsters receiving the test materials; this is most notable in the higher quartz dose groups. The confounding effect of this disease process on the expression of pulmonary fibrosis has yet to be evaluated.

TABLE 2: Body Weight Data Summary
Week 55 (Postexposure Week 40)
December 23, 1980

Group	No. of Animals	Age (Days)	Mean Body Weight (g)	Standard Deviation
Quartz, 6.0 mg	14	453	139.79	11.37
Quartz, 3.3 mg	16	453	141.50	11.04
Quartz, 0.33 mg	21	453	138.67	22.84
Quartz, 0.03 mg	19	453	138.74	13.29
Quartz + Fe_2O_3, 6.0 mg ea	12	453	127.92	12.80
Quartz + Fe_2O_3, 3.3 mg ea	16	453	136.44	19.74
Quartz + Fe_2O_3, 0.33 mg ea	24	453	139.42	20.42
Quartz + Fe_2O_3, 0.03 mg ea	20	453	139.40	20.25
Glass, 10.0 mg	21	453	136.67	14.20
Glass, 1.0 mg	22	453	131.55	19.20
Glass, 0.5 mg	22	453	143.95	20.21
Glass, 0.05 mg	19	453	131.58	17.96
Alumina, 20.0 mg	19	453	132.84	18.03
Alumina, 5.0 mg	19	453	143.53	18.69
Alumina, 2.0 mg	17	453	142.18	13.55
Alumina, 0.2 mg	19	453	138.16	16.12
Saline Controls	20	453	143.75	17.10
Cage Controls	25	453	146.56	14.64

TABLE 3: Body Weight Data Summary
Week 81 (Postexposure Week 66)
June 23, 1981

Group	No. of Animals	Age (Days)	Mean Body Weight (g)	Standard Deviation
Quartz, 6.0 mg	0			
Quartz, 3.3 mg	0			
Quartz, 0.33 mg	17	635	126.82	25.58
Quartz, 0.03 mg	14	635	133.71	17.11
Quartz + Fe_2O_3, 6.0 mg ea	0			
Quartz + Fe_2O_3, 3.3 mg ea	5	635	119.80	10.95
Quartz + Fe_2O_3, 0.33 mg ea	19	635	136.58	18.19
Quartz + Fe_2O_3, 0.03 mg ea	15	635	132.60	11.52
Glass, 10.0 mg	17	635	142.06	17.29
Glass, 1.0 mg	13	635	137.15	23.41
Glass, 0.5 mg	13	635	148.00	20.66
Glass, 0.05 mg	13	635	129.00	16.39
Alumina, 20.0 mg	12	635	127.25	23.46
Alumina, 5.0 mg	16	635	136.63	17.61
Alumina, 2.0 mg	8	635	142.13	11.98
Alumina, 0.2 mg	9	635	140.67	11.84
Saline Controls	14	635	136.86	20.94
Cage Controls	18	635	141.28	13.56

TABLE 4. NIOSH Fibrosis Study - Survival Rates

Group	Total Animals	Effective Animals*	SURVIVAL, WEEKS										
			0	5	10	15	20	30	40	50	60	70	80
Quartz													
6.0 mg	25	25	25	25	25	23	21	21	20	16	8	0	0
3.3 mg	25	23	25	24	23	21	21	20	19	18	13	6	0
0.33 mg	25	25	25	24	23	22	21	21	21	21	19	18	16
0.03 mg	25	24	25	24	22	20	20	20	20	19	19	17	13
Quartz + Fe_2O_3													
6.0 mg ea	25	26	25	23	23	22	21	20	16	12	7	0	0
3.3 mg ea	25	25	25	22	22	22	21	20	18	16	12	10	0
0.33 mg ea	25	25	25	24	24	24	24	24	24	24	23	21	19
0.03 mg ea	25	24	25	24	23	23	23	23	22	21	20	19	15
Glass													
10.0 mg	25	25	25	25	25	24	23	23	22	22	20	20	17
1.0 mg	25	25	25	24	24	23	23	23	22	22	18	16	13
0.5 mg	25	25	25	24	24	24	24	23	22	22	21	21	13
0.05 mg	25	25	25	24	24	24	24	20	19	19	18	13	13
Alumina													
20.0 mg	25	24	25	24	23	22	22	22	22	19	19	15	12
5.0 mg	25	25	25	24	24	23	22	20	20	19	18	16	16
0.5 mg	25	24	25	24	23	22	22	21	20	17	14	12	8
0.05 mg	25	25	25	24	23	23	23	22	22	19	17	12	8
Saline													
0.5 ml	25	23	25	23	22	22	22	21	21	21	20	15	13
Cage Controls	25	25	25	25	25	25	25	25	25	25	25	23	17

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*Effective animals = 25 - # of technique or anesthesia - related deaths + # of animals replaced/group

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DR. YODAIKEN: What prompts the choice of animal?

DR. LEWIS: Okay, our choice was the rat and the hamster. We chose the hamster because it is freer of pulmonary diseases. We thought we would have a greater likelihood of animals surviving throughout the duration. We also had the Saffiotti model for the ferric oxide. I hope it is the right choice. The converse is the rat where you get cancer more peripheral in the lung parenchyma, whereas, the classic cancer that you see in the hamster are in the broncheal area. But I am hoping that wherever you see fibrosis and you challenge with the cancer, you will get it.

PROCEEDINGS OF THE
SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Extent-of-Exposure Study of Wood Preservatives

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Extent-of-Exposure Study of Wood Preservatives

This is a study of industrial hygiene characterization of wood preservatives in wood treatment plants. This study was conducted by contract for NIOSH. Steward-Todd Associates Inc. being the contractor. The principal investigators were Allen Todd and Cindy Timbie.

Basically, NIOSH has the responsibility to conduct what we call "extent of exposure" studies, which define and detail what is really going on in the work place as far as the occupation and the worker exposures in industry.

This is one study; one of many of this type of study that is presently ongoing both in-house and under contract.

The major wood preservative chemicals that were studied and are in use in industry today include: (1) creosote, used as 100 percent creosote or as used in solution of 50 percent, or possibly less, along with petroleum oil; (2) pentachlorophenol, used as a 5-10 percent solution in a light to heavy oil, or it can be used in volatile solvents, LPG gas, or methylene chloride. There are several water-borne materials that are used. Two of which are most popular include Chromated Copper Arsenate (CCA) and Amonia Copper Arsenate (ACA).

There are other preservatives which are also used to a much lesser extent. These include such water-bornes as the Fluoro Chrome Arsenate Phenol (FCAP), the Acid Copper Chromate (ACC), the Chromated Zinc Chloride (CZC), Copper Napthylenate, Copper -8- Quinolinate, and Tributyl TIN Oxide (TBTO).

The breakdown on the usage of three principal materials is given in Table 1. For example, the railroad industry uses creosote for 97 percent of the railroad ties, treated. This is because of the various properties that it instills. Creosote is a primary use also for marine pilings (82%), and is used to a lesser extent for telephone poles (28%) and fence posts (24%).

The pentachlorophenol is principally used for telephone pole treatment (66%) and to a lesser extent for such things as fence posts (54%). The arsenicals, CCA, ACA and FCAD, are used predominantly for dimension lumber (70%) and to a lesser extent for fence posts (22%) and other uses.

The occupational exposure limits for the chemicals of concern are listed in Table 2. It is important to note that many of these are readily absorbed through the skin, and skin contact with these materials is an important exposure aspect.

In this study, 13 treatment plants selected for study in the preliminary walk-through phase, and four of these were then selected for more detailed, comprehensive study, involving personal sampling of workers.

Table 3 shows the summation of the sampling data collected at the four facilities in a comprehensive study. The 8-hour time-weighted average exposure levels (TWA), as well as the task oriented exposures, are all less than the recommended occupational exposure limits. Singularly high task level of 1670 and 3300 ng/m^3 are reported for creosote and arsenic, respectively.

Typically, the treatment of lumber is conducted at a lumber yard where lumber is preseasoned and stacked in the yard prior to treatment.

PROCESS DESCRIPTION

The thermal process of treating telephone poles is typically performed in an underground rectangular tank filled with hot treatment oil.

First, the tank is filled with poles and locked in place with lids loosely fitted to the top of the tank. The treatment material is brought into the tank and drives out what moisture is in the wood. The chemicals are heated to perhaps a little more than 200 degrees, and then reduced in temperature to around 120. It is in this cooling process that the treatment chemicals are absorbed in the open pores of the wood. The tank is drained.

Utility company men take borings on the poles prior to emptying the tank. The borings are inspected for penetration analyses before the poles are removed.

Similarly, a butt treatment tank is used where simply the ends of the telephone poles are treated either with penta or creosote. This is an open dip tank process. There are often vapors actually coming off these tanks.

Actually, about 98 percent of the wood that is treated in this country is treated in pressure cylinders. These are above ground. A typical wood charge is stacked on the tram care and pushed with an engine into the open cylinder. The loading of a cylinder with a charge presents minimum operator exposure. Basically, the charge is simply pushed with an engine into a cylinder. The wood is very carefully stacked on the rack so that they just fit, and they can get the maximum use of the treatment process.

The back end of the cylinder is tied into the pump room and system control room. The control panel area is where the treatment operator spends most of his time. There is, of course, limited exposure in this area unless there would be servicing or maintanance of equipment, or an accidental spill of chemicals.

The other end of the treatment cylinder, this cylinder door, may operate manually or as an automatic hydraulic door. The manual method permits greater exposure for the operator.

The hydraulic door is a substantial improvement. It does not have the bolts. It is operated automatically and does not require the manual tightening of the bolts. Often at the bottom of the door, some bilge water is common at the end of the cycle. After the treatment is finished, the pressure is released on the door and what remains in the bottom of the tank often is drained into a drain system which is recycled into use.

Once the door is opened, the rail is dropped and the unloader at this point has to pick up a cable and attach it to the front end of the engine which then proceeds to pull the load out. This is the time when there is potential for exposure. And this is evidenced by the vapor coming off the top of the tank.

SUMMATION

All of these processes that involve possible exposure, personal contact, we would like to control to the extent possible, using protective clothing, such as disposable coveralls or other appropriate work clothing. And this should be used for any time, or any employee that has to go into the cylinder for any reason, and also for any yard personnel assisting in unloading of the cylinder or restacking the treated material. Gloves can be used if they can be used safely to help minimize hand and arm contact with the treated wood. There should also be appropriate facilities for change rooms, showers, wash up facilities, and separate eating areas.

In conclusion then, I would like to say about the treatment industry that basically we are talking about a small work force, about 500 treatment plants in this country and generally about four to 15 workers that are actually exposed at each operation.

The finished products do contain potential carcinogens, the arsenic and creosote and penta material particularly. And of greatest concern for these, of course, is the individual's contact with them, the exposures that he may pick up quite accidentally in coming in contact with them at a plant.

I think that it is appropriate to stress primarily two methods of controlling these exposures. The first is, of course, work practices and the importance of work practices. The second is medical surveillance because basically we are dealing with exposures to chemicals which are absorbed through the skin and cannot be adequately evaluated by breathing zone or air sampling techniques.

So it is quite important that we include biological sampling at these plants. Thank you very much.

TABLE 1

MAJOR USAGE, % TOTAL

	CREOSOTE	PENTA	CCA/ACA/FCAD
RR Ties	97	0.5	2.5
Poles	28	66	6
Pilings	82	10	8
Lumber	10	20	70
Posts	24	54	22

TABLE 2

OCC. EXPOSURE LIMITS

	TWA(UG/M^3)	STEL (UG/M^3)
PCP (SKIN)	500	1500
CREOSOTE (CTPV)	200	-
ARSENIC	200	-
COPPER MIST	1000	2000
CHROMIUM (III)	500	-
CHROMIUM (VI)	50	-

TABLE 3

4 PLANT COMPREHENSIVE WP STUDY
PERSONAL AIR MONITORING SUMMARY
(UG/M^3) MEDIAN/HIGH LEVEL

AGENT	NO. OF SAMPLES	TWA	TASK*
CREOSOTE	8	21/60	
	18		28/1670
PCP	3	13/19	
	24		25/275
ARSENIC	-	-	
	8		ND/3300
CHROMIUM	-	-	
	4		ND/6
COPPER MIST	-	-	
	8		ND/69

NOTE:

* TASK ORIENTED REFERS TO SAMPLES OF LESS THAN 300 MINUTES DURATION

GRAVEMETRIC ANALYSIS RESULTS REPORTED FOR CREOSOTE

N.D. INDICATES BELOW DETECTABLE LIMIT

DR YODAIKEN: Thank you very much. We have time for one question perhaps. One quick question? Thank you.

DR. BLOT: Bill Blot, from NCI. Is there a potential for exposure to arsenicals from home or garden use of lumber ties or lumber products?

MR. OSER: That is a good question. There would be, indeed, if these chemicals are free, you know, within the wood. There is some question as to whether or not they would be at that time after several rains. There is potential for arsenic exposure at the treatment plants. We have taken samples and can determine the presence of arsenic in those samples. So there is a potential. Now, the extent to which that is carried through to home use, you know, that is a difficult question.

SPEAKER: Were there any measurements for dioxin in pentachlorophenol?

MR. OSER: We actually took no air samples in terms of breathing content. But we did sampling of the wood itself. And there are dioxins in the pentachlorophenol, which we are aware of.

SPEAKER: I have just one quick question. He talked about the process being fairly localized but when they drain that tank it looks as if there is material going off into an open pit for recycling purposes. Is there a problem with that going back to a system or is it a fairly short distance between that trough and where it goes back into closed pipes? That is an exposure question I would like to hear an answer to.

MR. OSER: Well, yes, there is potential for exposure there. That is one of the points that we are going to check. There are fumes, there are vapors coming off. But generally, this is minimal, the extent of this exposure is minimal.

SPEAKER: But part of this is not going down some sewer system somewhere?

MR. OSER: We hope not.

SPEAKER: In other words, you haven't looked yet into that issue?

MR. OSER: We really have not evaluated that issue.

PRCCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Carcinogenesis of Roofing Asphalts, Pitch and Simulated Sunlight

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CARCINOGENESIS OF ROOFING ASPHALTS, PITCH AND SIMULATED SUNLIGHT

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ABSTRACT

The tumorigenicity to mouse skin of volatile components of roofing asphalts and coal tar pitches has been investigated in an 18-month experimental program. The primary factorial experiment involved 32 groups of 50 mice each. The experimental variables were: two strains of male mice (CD-1 and C3H/HeJ); four initial roofing materials (asphalt, Types I and III; coal tar pitch, Types I and III); two temperatures of generation of volatile fume materials (232° and 316°C); and exposure to simulated sunlight vs. the absence thereof. Treatment with condensed volatiles and light was performed twice weekly. Additional groups were a combination treatment (alternate weekly application of 316° volatiles from Type III asphalt and Type I coal tar pitch); a positive control (benzo(a)pyrene, with and without sunlight); and negative controls for solvent and sunlight.

Asphalt preparations were applied as 50% solutions in cyclohexane/acetone, providing 25 mg per mouse per application of 50 μL. Pitch preparations were diluted to produce a solution containing 0.01% benzo(a)pyrene which resulted in total solids concentrations of 3.0 to 8.4% in the various preparations, and application of 1.5 to 4.2 mg per mouse per application. All preparations were analyzed by GC/MS for selected polynuclear aromatic hydrocarbon (PNA) and heteroaromatic compounds.

The solar simulator consisted of an Atlas 6.5 kW xenon arc, appropriately filtered and mounted to provide one solar equivalent to the mice at the nearest point on two rotating turntables, enclosed in an air-cooled chamber.

Analyses of the results have led to the following conclusions:

> With the asphalt volatiles, there was a pronounced effect of temperature of preparation on tumorigenesis, the 316° preparation being the more active. The CD-1 groups averaged less than 0.3 month decrease in mean latency when exposed to the higher temperature fumes, but the total corrected tumor incidence increased markedly in the non-solar exposed animals by 2.6 and 1.5 fold for Types I and III asphalt fumes, respectively. The average tumor incidence for the CD-1 groups was 26.3 ± 11.7% ($\bar{x} \pm$ SD). The C3H/HeJ mean latency response to the higher temperature fumes averaged 1.9 months less than the groups exposed to the lower temperature fumes; however, there was little change in total corrected tumor incidence, which averaged 89.2 ± 6.3%, that could be attributed to the temperature of generation. This increase in tumorigenicity was accompanied by a decrease in the measured PNA content.

- Pitches did not show this effect, but since the 316° preparations were applied at lower concentrations and showed tumorigenicity equivalent to that of the 232° pitch preparations, the former are inferred to have higher specific activity. The average corrected tumor incidence for the C3H/HeJ groups was 96.0 ± 3.3% while that for the CD-1 groups was 69.5 ± 13.5%.

- The combination treatment group showed effects, as might be expected from the individual activities of the two 316° preparations used, without evidence of additivity or synergism.

- The male C3H mouse was much more sensitive than the male CD-1 mouse to the tumorigenic activity of the asphalt volatiles, in particular, but also of the pitches.

- Simulated sunlight, as used in this experiment, had an inhibitory effect on the rate of appearance of tumors and on the final tumor incidence. In some cases, there was little inhibition, but no marked enhancement. The mean latency period for C3H/HeJ groups treated with sunlight and asphalt or coal tar pitch fume increased 0.8 and 0.9 months, respectively, as compared to fume treatment alone; whereas the same comparison in the CD-1 strains showed a 1.5 and 2.3 months increase in mean latent period, respectively. The total tumor incidences in the C3H/HeJ groups were influenced little by the presence of sunlight; however, the CD-1 groups exposed to sunlight plus fume exhibited marked inhibition of tumor response especially those exposed to the high temperature asphalt (∿60%) and coal tar pitch (∿35%) fumes.

- Conversion ratios of tumor types, i.e., from benign to malignant, were high for the C3H/HeJ groups (∿68%) and much lower for the CD-1 groups (∿10%). These ratios were roughly equivalent for both types of materials. Benign tumors types included papillomas, kerotoacanthomas and unclassified epitheliomas; while the majority of malignant tumors included squamous cell carcinomas and some fibrosarcomas.

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INTRODUCTION

Significant increased risks in developing cancer of the lung, upper respiratory tract, and upper GI tract, including stomach cancer, have been demonstrated for those individuals working 20 or more years in roofing operations (Hammond, et al., 1976). These investigators have also documented trends of increased cancer risk at other sites, e.g., prostate, bladder, skin and leukemia in these workers. It appears that the occupational exposures, as incurred by roofers, are associated with high levels of polynuclear aromatic hydrocarbons (PNA) arising from the heating and application of petroleum asphalts and coal tar pitch. These findings are consistent with results of other studies which have indicated that excessive occupational exposure to PNA may be associated with increased mortality from various types of cancer. Examples of these occupations include chimney sweeps (Pott, 1775), mineral and shale oil users (Scott, 1922 and 1923; DeCoufle, 1976; Bingham, et al., 1979), gas-workers (Doll et al., 1972), and coke oven workers (Lloyd, 1971; Mazumdar et al., 1975; Redmond et al., 1972). Skin cancer mortality may be of borderline significance in many of these occupations only because deaths due to skin cancer are rare. However, skin cancer incidence may be excessive.

The principal source of coal tar pitch is the coke-oven plant where hot gases and vapors produced during the conversion of coal to coke are collected by a scrubber which condenses the effluent into ammonia, water, crude tar and other byproducts. Distillation of the coal tar produces a variety of compounds, including coal tar pitch which is the residual product. The grade of coal tar pitch produced depends on the retention time and temperature in the fractionating column (NIOSH, 1977b).

The carcinogenicity of neat coal tar pitch, as well as many of its components, particularly PNA, has been widely investigated and reviewed (NIOSH, 1977b). The carcinogenicity of aersolized coal tar has been liberally studied; however, the carcinogenicity coal tar pitch fumes as generated in roofing operations has not been investigated.

Asphalts are usually obtained as the residue of the distillation of petroleum crudes. Their chemical composition varies depending on the crude oil used to manufacture them, the refining process and the physical specifications of the finished product. Besides naturally occurring asphalt sources, asphalts are also derived from crude oil by solvent precipitation and air-blowing. The former process is used primarily to obtain either lube or catalytic-cracking feedstock oils, rather than for the production of asphalts. Air-blowing is a process for further refining of the asphalt stock where air at temperatures of 204 to 288°C is bubbled through the stock producing dehydrogenation and polymerization reactions. This process is generally used to manufacture paving and roofing materials. (NIOSH, 1977a).

The carcinogenicity of asphalts is not well documented primarily because of the confusion of many authors concerning the terms asphalt, pitch and tar (coal tar) which are frequently used interchangeably and incorrectly. Limited chemical analyses show the substances to be quite different, especially in their proportions of PNA and other known carcinogenic chemicals (Wallcave et al., 1971; Puzinauskas and Corbett, 1978; Bingham et al., 1979). Bingham and coworkers (1979) and NIOSH (1977a) reviewed the literature concerning the carcinogenic potential of asphalt. Bingham and coworkers concluded that "the potential for inducing cancer by petroleum-derived asphalts, which in certain instances is clearly less than that of coal tar products, is dependent in a complex way upon their source and is influenced by refining history and the processing of the final mixtures".

In addition to the risks associated with exposure to the petroleum asphalt and/or coal tar pitch fumes in the roofing occupational environment, the added risk of sunlight exposure in the production of cancer in this out-of-doors environment must be considered. It was highly suspect that exposure to sunlight may affect the skin cancer incidence in this population based on comments by Suskind (1974). Varicus investigators working with experimental animal models have observed that ultraviolet and/or visible light augments the carcinogenic end point of PNA exposure (Santamaria, et al., 1966; Urbach, 1959). The reverse may also be true, i.e., that PNA and/or associated alkanes contained in the pitch or asphalt fumes augment the carcinogenic end point of UVL exposure, i.e., act as cocarcinogens (Bingham and Nord, 1977). Santamaria and Giordano (1969) mentioned the conflicting results found in the literature where the action of UVL on PNA carcinogenesis resulted in acceleration, inhibition, as well as in no effect on cancer induction.

NIOSH estimates that there are 12,000 roofing contractors employing over 116,000 workers in the U.S. Since these workers have combined exposures to asphalt and coal tar pitch fumes and sunlight, a complex 18 month experimental study was designed to determine the relative importance of exposures to each and combinations of these agents.

The purpose of this investigation was fourfold. The first objective was to assess in the animal model the carcinogenic potential of condensed volatiles from roofing asphalts and coal tar pitch materials that were collected from fumes generated at the recommended application temperatures of the materials.

Since the heating process in the kettle operations is usually not controlled, it is common for the materials to be heated above their recommended application temperatures (Thomas and Mukai, 1975). The excessive heating is known to result in pyrolysis of the materials, leading to an increased production of PNA. Therefore, the design of this study incorporated a second objective: to assess the carcinogenic potential of the condensed volatiles collected from fumes generated at temperatures in excess of their recommended application temperatures.

The third objective was to assess the effects of concomitant exposure to simulated sunlight on the carcinogenic outcome of the above materials.

Because of the expected differing results from exposure to simulated sunlight a fourth objective was included: to assess the responses in a pigmented stain of mice and compare this to the responses in a nonpigmented stain.

MATERIALS AND METHODS

There are four types of asphalt, Types I through IV, and three types of coal tar pitch, Types I through III, used for roof dampproofing and waterproofing. Only two types of each were used in this study and they were chosen on the basis of common use and extremes of the classifications. These are Type I and Type III asphalt, referred to in the industry as "dead level" and "steep", respectively, and Type I and Type III coal tar pitch often referred to as "regular roofing" and "low fuming" pitch, respectively. These four materials are produced by several manufacturers according to physical specifications recommended by the American Society of Testing and Materials (ASTM). A description of the materials and their temperature specifications are given in Table 1.

Approximately 270 kg of each of the four materials were purchased to provide the necessary volume of skin painting solutions for the bioassay. The asphalts, purchased from a local distributer of Exxon, Inc., were produced by distillation and air blowing of Arabian crude and met ASTM specifications. Type I pitch was provided by Reilly Tar and Chemical Corporation and Type III pitch was provided by Koppers, Inc. Both materials were manufactured to ASTM specifications and were shipped from available inventory.

The collection of fumes from a roofing material kettle in the field would be awkward at best due to the difficulty in controlling sample mixing, temperature, exposure to sunlight and in collecting a condensed sample at a subambient temperature. Therefore, laboratory generation of fumes from an easily controlled glass generation system and collection of condensed material in a glass cryogenic system was used for production of necessary amounts of fumes. A 12 L round bottom reaction flask was used to contain about 10 L of roofing material during individual fume generations. The roofing material was broken into smaller pieces by a chisel or cut with a warm knife and placed into the weighed 12 L flask. The flask and its contents were warmed in a forced air oven at 150° C and, as the material melted, more was added until approximately a 10 L volume was achieved. Between zero and 30° C above the softening point a stainless steel multifinned stirring rod, operated by an air pump, was inserted through a 3-hole reaction flask head with a Teflon stirrer gland to permit uniform mixing (250 to 300 rpm). An electric heating mantle capable of reaching 450° C was utilized to heat the roofing material to the desired generation temperatures. Mantle temperatures were controlled to maintain the system to within 5° C of the desired generation temperature. Nine thermocouples were arrayed within the generator to monitor the temperatures during a profile run. These were replaced by only one thermocouple during the actual collections.

The fume collection system consisted of glass transfer tubes (20mm OD) and 500 mL glass impingers (Ace Glass, Inc.) placed in three individual cryotraps containing various media, i.e., ice, (0° C), dry ice/isopropanol (-77° C), liquid argon (-186° C). An additional impinger containing a 50/50 mixture of cyclohexane/acetone was used after the cryotraps to provide additional collection through dissolution. After the first few generations, a second dry ice/isopropanol bath was used to replace liquid argon because of cost considerations. The impact of this change was determined for Type I asphalt and Type I pitch at 232° C and 316° C. No change in breakthrough from the cryogenic trap compared to the second dry ice trap was observed. A glass fiber filter was occasionally placed downstream of the impingers to permit an indication of breakthrough of particulates; however, the material on the filter was never added to the total fume sample. Amounts collected on the filter were generally less than 0.3% of the total amount of material collected.

Air was cleaned using a high efficiency filter (Filterite 0.45 µm Microflow cartridge), silica gel and granular activated charcoal for removal of particulates, water and organic vapors prior to entering a rotameter for flow monitoring and then a tube furnace to preheat the air to about 100° C. The preheating prevented premature condensation of fumes on the reaction flask wall. The air was then pulled through the reaction flask where it entrained volatiles and then into the cryotraps. Clean, dry air was pulled through the system at a rate of 10 LPM with a vacuum pump (Gast, Inc.) regulated by needle valve restrictor (Hoke, Inc.). The generation-collection system was contained in a laboratory which received only yellow light filtered through cellulose acetate-butyrate filters to reduce exposure to ultraviolet light.

Fumes were generated at approximate field kettle temperatures for each material and a high overheat temperature which was just below the flashpoint of the material. Temperatures observed in the field for each material are given in Table 1. Note that the normal kettle temperature may be up to 28° C higher than the temperature desired at the point of application. The lower generation temperature for all materials in this study was 232° C which represents a kettle temperature frequently observed in the field. This temperature is somewhat low for steep asphalt but makes the temperature difference between the laboratory simulated kettle temperature and overheat temperature (316° C) equivalent for all materials. It was not apparent from the literature that one type of material would be overheated to a greater extent than another in the field.

After the fumes were condensed and collected in the sampling train and individual impingers and transfer lines were weighed, all material was quantitatively transferred to a large flask with an excess of cyclohexane/acetone solvent mixture used to assist complete transfer. Sufficient solvent was added to ensure complete solution of the collected fumes. If a water phase existed, it was removed by transferring the entire solution to a separatory funnel. The organic phase was transferred to a rotary evaporatory (Beuchler, Inc.) and the solvent removed at reduced pressure with a water aspirator and at a temperature of 50° C. The solvent was discarded after gas chromatographic/mass spectrometric (GC/MS) confirmation was obtained that no significant amounts of compounds of interest were present. The aqueous phase, when present was transferred to an evaporating dish and the water removed in a vacuum oven at 300 mm Hg and 50° C. The materials (fumes) remaining after removal of solvents (water and cyclohexane/acetone) were weighed and dissolved in a 50/50 (v/v) cyclohexane/acetone mixture and combined. The choice of this solvent for collection, rinsing and preparation of skin painting solutions was the result of the following rationale: (1) observations of the poorer solubility of roofing material fumes in either solvent alone; (2) the low boiling point of each solvent; (3) the compatability of each solvent with respect to PAH stability; (4) the lack of absorption of light in the simulated sunlight range; and (5) the inactivity of each with respect to mouse skin bioassays. The relative distribution of fumes in the collection system for some of the materials is given in Table 2.

A total of one hundred forty-four successful daily generations were conducted to provide sufficient condensed material from the heating of four different roofing materials at two different temperatures to permit the preparation of about four liters of eight skin painting solutions. While only one or two generations were necessary to provide enough pitch volatiles for each temperature, 59 generations of Type I asphalt at 232° C, 6 runs for Type I asphalt at 316° C, 34 for Type III asphalt at 232° C, and 7 generations for Type III asphalt at 316° C were required. Where possible, several generations were combined into a single batch. Although it was desirable to use only one batch of each solution, the slow generation of asphalt volatiles required preparation of many individual batches over the duration of the skin painting experiments. Each batch was analyzed for selected PNA and heteroaromatic compounds content to obtain a fingerprint of the relatively important chemicals.

The total mass of volatiles emitted during heating of roofing materials may differ significantly over a temperature range from a typical kettle temperature to a high overheat temperature and also between the different types of asphalts and pitches. Since both the dose and the specific carcinogenicity of a material are generally important in determining the health hazard each presents, the mass emission rate of volatiles during laboratory generations may be of interest as a simulation of field emission rates. The emission rate during each generation was simply calculated from the mass of volatiles collected in a given time period. The data summarized in Table 3 indicate the marked increase in the mean mass emission rate at the elevated temperature. The emission rate for asphalts is generally about ten times higher at 316° C than at 232° C. In the case of coal tar pitches, the emission rate is about two to four times greater at the higher temperature. The emission rate is also much greater for the coal tar pitches.

To determine the concentrations of selected PNA in skin painting solutions, combined gas chromatographic/mass spectrometric (GC/MS - Finnigan Model 4023) analysis was used. A glass capillary column (25-m coated with SP-2250) capable of high resolution was generally employed to permit separation and quantitation of closely related isomers such as benzo(e)pyrene and benzo(a)pyrene. The GC temperature program for the analyses was the following: 30° C isothermal for 2 min; 30-175° C at 20° C/min; 175-285° C at 3° C/min; and 285° C isothermal for 30 min. The MS conditions were: full mass scan mode, 100 to 350 amn, 2 sec/scan, 50 eV, filament emission of 45 ma and electron multiplier voltage of -1900 v. Compounds were considered to be identified when the retention time for the chemical of interest relative to the retention time of an internal standard, i.e., d 10-anthracene, 9-phenylanthracene or 9,10-diphenylanthracene, matched the relative retention time observed in a calibration standard, and the mass spectrum of the PAH of interest matched that obtained from a calibration standard. The concentrations of 18 selected chemicals for the eight skin painting solutions are given in Tables 4 and 5.

A solution containing 0.01% B(a)P was desired for all four roofing materials, as long as the total solids concentration did not exceed 50% w/v. In the case of the coal tar pitch fumes, sufficient B(a)P was present to require dilution to prepare a solution containing 0.01% B(a)P. The level of solids present in the coal tar pitch fume solutions was less than 10% (Table 6). However, in the case of the asphalt fumes, less B(a)P is present, and all asphalt solutions were prepared at the 50% w/v level. Also indicated in Table 6 are the designations used as test codes in the bioassay laboratory. These solutions were stored in brown glass bottles at 4° C prior to use for skin painting.

The solar simulator arrangement developed for this study used a 15 cm Atlas 6.5 kW Xenon arc, water cooled, quartz enveloped burner that was located about 53 cm above midway between two turntables (Figure 1). The light control-turntables were mounted directly below those receiving the light. The turntables rotated at two revolution per minute. The exposure cages, described below, were located on the turntables. Short wavelength ultraviolet below 290 nm was blocked using two IR-reflecting, metal oxide coated, 3 mm tempax glass, No. I14 Shott filters (Maing, W. Germany) mounted on a framework immediately adjacent to the light source. A silicon detector (UV-215B, EG&G, Salem, MA) with an opal glass diffuser, was connected to a counter which integrated the irradiance. Light measurements were made to validate the geometric concept of the turntable arrangement. Another silicon photodiode detector, spectrally calibrated from 200 to 1100 nm, was mounted in a holder that would accept one of five calibrated narrowband optical interference filters including 309 nm, 340 nm, 410 nm, 570 nm, 600 nm and 790 nm. Measurements were obtained at five positions (Figure 1) with the five filters to assess the spectral irradiance distribution. These measurements were repeated periodically throughout the study to assure uniform performance of the solar simulator. An automatic exposure control detector was included in the design which maintained a constant exposure by adjusting the exposure time to correct for instantaneous lamp intensity. This integrating system eliminated the effects of fluctuations and slow changes in intensity of the arc due to aging.

Two enclosures, one containing the light source and another surrounding the light source and the turntables were constructed of aluminum (former) or aluminum framing covered with sheet masonite (latter). Overall dimensions of the enclosed space were 2.1 m wide x 1.2 m deep x 1.5 m high. Air was exhausted through the top at approximately 8.5 m^3/min. (300 cfm), with the incoming air led from the open bottom, through baffles placed to maximize ventilation of the test cubicle arrays. Air temperature in the box was thus kept in a range between 24 and 28° C (75-80° F). There was no olfactory evidence of the production of ozone. This shielding of the light source was designed to prevent exposure of personnel and effectively preclude additional exposure of the experimental animals.

The goal of the experiment was to produce tumor onset in light exposed control group at approximately 50 weeks. From Bingham and Nord (1977) and Burns (1978) it was estimated that a total UVB dose (280-320 nm) required to produce this effect was approximately 2×10^5 Wsec/m^2 over 100 sessions of exposure. Thus, the required duration of exposure per session based upon the available data was approximately 49 minutes.

The male mice used in these bioassays were of two strains: non-pigmented Swiss CD-1 (Charles River) and pigmented C3H/HeJ (Jackson Laboratories). Upon arrival, at six weeks of age, all animals were quarantined for a 6 to 9 week period. Animals were housed individually in stainless steel suspended metal cages and provided food and water ad libitum except during the exposure period. Each test group consisted of 50 formally randomized mice of a given strain. Each mouse was identified by toe-clipping and an ear puncture system served to identify mice by experimental group. The 48 experimental groups included:

a. 32 groups for the primary factorial experiment, i.e., 2 strains x 4 materials x 2 generation temperatures x 2 light exposure conditions (presense or absence of simulated sunlight)--Groups 1 through 32

b. 4 groups for the solvent control, i.e., 2 mouse strains x 2 light exposure conditions--Groups 33 through 36

c. 2 groups, i.e., 2 mouse strains, for a cage control receiving only solvent and not sham irradiated but always maintained in their individual cages--Groups 37 and 38

d. 4 groups for the positive control (0.01% BaP in cyclohexane:acetone (1:1)), i.e., 2 mouse strains x 2 light exposure conditions--Groups 39 through 42

e. 4 groups receiving a combination treatment of asphalt and coal tar pitch fume condensate, i.e., 2 mouse strains x 2 light exposure conditions--Groups 43 through 46

f. 2 groups receiving no skin painting treatment but light exposure only, i.e., 2 mouse strains--Groups 47 and 48.

All odd numbered groups were CD-1 mice and even numbered groups were C3H/HeJ mice.

Animal racks were regularly rotated within the animal quarters, and to minimize the potential effects of fluorescent lights lamps were enclosed in filter tubes (Crown Plastics Corp.). The automatic light cycle in the animal quarters was adjusted to 12 hours for each light and dark period. Each mouse was weighed prior to test commencement and prior to each first weekly application for six weeks, and biweekly thereafter.

hair was carefully clipped from the interscapular region of each animal prior to treatment, as needed, using a small animal electric clipper (Mode A-2 Oster Co.). A separate clipper head (size 40) was used for each test material. Fifty µl of each test material was applied twice weekly to each mouse of a test group using disposable tip automatic pipettes. After chemical treatment, each mouse of a group was placed in a cell (4 x 9 x 3 cm) of a clean exposure cage unit which accomodated the entire group of 50 mice. These units were specially designed to fit the round turntable. The cage unit was constructed of 3 x 3 stainless steel mesh with reinforcement bars and sliding mesh tops.

Each mouse in groups 1 through 32 received 50 µl of the appropriate test material twice weekly. Each animal in the negative (1:1 cyclohexane/acetone) control group received 50 µl of the vehicle twice weekly. The positive control group animals received 50 µl each of 0.01% benzo(a)pyrene (5 µg of benzo(a)pyrene) in 1:1 cyclohexane-acetone twice weekly. Benzo(a)pyrene was obtained from the NCI Chemical Repository (IITRI, Chicago, IL). Cyclohexane and acetone were both HPLC grade (Fisher Scientific, Inc.). The combination groups were treated twice weekly on alternate weeks with 50 µl of the high temperature condensates from the Type III asphalt and the Type I coal tar pitch fumes.

Test material handling and administration were done in a ventilated hood. At 30 minutes following the application of the appropriate test material to the last mouse in the array, the group was exposed to the simulated sunlight. Two units were positioned on each of two turntables for solar exposures. For the nonsolar exposure groups, the array units were placed on lower-level platforms protected from irradiation. The foregoing confinement procedure was used for the non-irradiated, as well as, irradiated animal groups. To insure the long-term uniformity of exposure to the light source, the animals were rotated weekly into different cubicle locations on the unit by a formal procedure involving pattern assignment.

Mice were observed daily for mortality, evidence of systemic toxicity, and gross appearance of tumors. The mice in a specific group were treated and observed until 85% of a group died, or until 18 months had elapsed. Mice found dead were necropsied. Those that were moribund were killed and necropsied and when groups were killed, those remaining animals were necropsied. All tissues mentioned in the NCI guidelines were examined, collected and preserved in formalin. Gross diagnosis of a skin carcinoma was based on a lesion that upon palpation was attached to underlying tissues, which generally indicated invasion of connective tissue or muscle layers. All tumors were excised and fixed in buffered 10% formalin for microscopic examination. The distribution of tumors between dermal sarcomas, papillomas and epidermal carcinomas was noted particularly.

RESULTS

In general, survival of all groups of mice was satisfactory and comparable through 11 months for CD-1 mice and 10 months for C3H/HeJ mice. Survival was greater than 80% for all groups at the appearance of the first papilloma (effective number of animals at risk) for the respective groups except in groups

3 and 5, i.e., CD-1, Type 1 asphalt at 232° C, solar exposure and CD-1, Type 1 asphalt at 316° C, solar exposure, respectively (Table 7). Both of these groups suffered major losses of animals when the ventilation system in the solar simulator failed temporarily on the 95th exposure (11 months) and the temperature within the chamber rose to over 40° C. After 10 to 11 months, the experimental groups showed sharper declines in survival because of termination of moribund animals to ensure preservation of tumors for histopathological examination. The control groups of both strains showed good survival throughout the study, approximately as expected. However, there were some excess deaths in the CD-1 controls, related to an endemic incidence of urinary tract infections.

Animal weights reflected very little effect of the treatments on weight gain. The major weight gains occurred in the first two months, and after that there were only minor oscillations. The effect of sacrificing moribund or cachectic animals is a factor in these oscillations.

Gross pathological examination of all necropsied animals revealed significant incidences of abnormal conditions in liver, spleen and lung (Table 8) in the treated compared to the controls. Some of these changes, however, appeared to be associated with aging, since they occurred in control groups as much as in groups treated with pitch or asphalt volatiles. The latter was particularly noted in CD-1 mice with pale livers, and apparent liver and lung tumors. Some of the gross pathologic effects, since they were observed to equivalent extents in B(a)P controls and volatile-treated groups, may be attributable to a generalized effect of PNAs. These generally include larged livers in the CD-1 strain, and in both CD-1 and C3H/HeJ enlarged and mottled spleens. Only one organ pathology, apparent liver tumors in the C3H mice, had a suggestion of lower incidence in both the combination and pitch groups, compared to B(a)P and negative controls. It is reasonable to assume that these groups may have experienced more liver tumors if survival had been greater; however, since many of the mice were sacrificed or died early because of skin tumors, the incidence was lower than expected. significance of lung tumors in the C3H/HeJ mice must be determined from future histopathologic examination.

Tumor incidence, both as observed grossly during the life of the animals and histopathologically, is presented in detail in Table 9. Pooled data derived from Table 9 are graphically presented in Figure 2. Mean time to tumor data are given in Table 10 with a graphic presentation of these pooled data in Figure 3.

Cumulative tumor incidence for all groups except the negative and cage control groups are presented graphically in figures 4 to 13.

From the pooled incidences of tumor-bearing animals expressed as percentage of effective total, both for total and malignant tumors (carcinomas or fibrosarcomas), given in figure 2 show that the CD-1 mice have a moderately high total tumor incidence, but quite a low incidence of malignant tumors, and hence low conversion ratios. Although the asphalts produced a much lower tumor incidence in the CD-1 as compared to the C3H/HeJ strain, the conversion ratios were comparable for both asphalts and pitches. With the C3H/HeJ mice, values were quite high for all three measures of effect. These data thus show the high activity of pitches on both strains of mice and of asphalts on the C3H only.

The time-incidence data have been used for calculation of latent period (mean time to tumor). These data (Table 10 and Figure 3) show the lower sensitivity of the CD-1 mouse strain, with longer latent periods than the C3H mice for all test materials except B(a)P. The mean increment (excluding B(a)P groups) is 2.2 ± 1.1 months. The increment is less than a month for only two groups (test materials A and E, nonsolar). The inhibitory effect of the simulated sunlight is manifested in these data, since in every pair but one (test material D on CD-1 mice) the latent period for the solar groups is longer than for the corresponding nonsolar group. This is pronounced with the CD-1 mice for which the mean increase (excluding D) with solar treatment is 2.1 ± 0.7 months, compared to 1.0 ± 0.6 months for the C3H's.

Analysis of data on tumor multiplicity of grossly observed tumors (data not shown) is another useful measure of carcinogenic activity. In the C3H mice, all treatments, except B(a)P, produced an average of more than 2 tumors per tumor-bearing animal, and the overall average was 2.4 (1986/749). The treated CD-1 mice averaged 2.1 (850/404), with only the nonsolar pitches and B(a)P groups being at or above 2.0. On examining the multiplicity data (not shown) for the combination experiment, in comparison with the two separate materials used, D and F, the combination treatment gave a higher degree of multiplicity than the individual components with the C3H/Hej mice, but this was not seen with the CD-1 strain.

CONCLUSIONS AND DISCUSSION

Examination of the data have lead to the following conclusions:

1. With asphalt fume condensates there was a demonstrable and sometimes pronounced effect of temperature of generation on tumorigenesis. The higher temperature (316^{o} C) being more active (Table 9 and Figures 4 through 7).

2. Coal tar pitch fume condensates did not exhibit the same temperature dependant effects as the asphalts; however since the 316° C preparations were applied at lower concentrations and showed equivalent tumorigenicity to that of the lower temperature preparations, the former are inferred to have higher specific activity (Table 9 and Figures 8 through 11).

3. There appeared to be few differences in the same animal strain as a result of exposure to condensates of the two different types of asphalt or coal tar pitch materials generated at the same temperature. The Type III asphalt fumes generated at 232° C were more active than the Type I asphalt at the same temperature in the CD-1 strain. Type I pitch at 232° C was slightly less active than Type III pitch generated at the same temperature and tested in the C_3H strain. Type I pitch at both temperatures was slightly more active than the Type III pitch in the CD-1 strain (Table 9 and Figures 4 through 11).

4. The combination groups receiving different treatments in alternating weeks show effects as might be expected from the averaged activity of the 316° pitch and asphalt preparations used (Table 9 and Figure 12).

5. The male C3H mouse was much more sensitive than the male CD-1 mouse to the tumorigenic activity of the asphalts, in particular, but also of the pitches. Relative incidence of malignant tumors was also low with the CD-1 mice.

6. Simulated sunlight, as used in this experiment, had an inhibitory effect on the rate of appearance of tumors, for the most part, and to some extent on the final tumor-incidences, particularly in the CD-1 groups.

A number of questions are raised by these experimental findings, which we believe should be investigated in order to further assess the risk of such products, particularly to roofing workers.

The asphalt volatiles were strikingly more tumorigenic and carcinogenic to the C3H mice than we expected from previous studies. The CD-1 mice gave results more in line with expectation, showing high activity of the pitches, and low activity of the asphalts. These differences raise several related questions:

1. Is there something unique about the C3H mouse? It is known to have higher levels of inducible arylhydrocarbon hydroxylase (AHH) than several other inbred (and hybrid) strains, but has apparently never been compared to random-breeds derived from the ICR-Swiss strain, like the CD-1.

2. Since the PNA content of the asphalt volatiles is very low compared to that of the coal tar pitch preparations, what other chemical components do these asphalts have which contribute to the observed carcinogenicity, as promoters, cocarcinogens, or even other carcinogens not of the PNA chemical class?

   This second point, regarding the differences in composition between pitch and asphalt volatiles, is further emphasized in the analysis presented in Table 11. The amount of total test material applied by the time of 50% tumor incidence (C3H mice) is very much larger for the asphalts, by factors of 6-22 times yet the amounts of total PAH analyzed or B(a)P are much smaller, by factors of 0.01-0.11 (PAH) and 0.005 to 0.04 (B(a)P). This prompts the suggestion that the asphalt preparations contain other materials, augmenting the activity of the PNA. Is it likely that methylated derivatives and nitro-PNA compounds account for some of this activity?

3. Linking the above two, is there some way in which the C3H mouse responds differently to some unique components of the asphalt materials, either in some cocarcinogenic or promoting sensitivity and/or sensitivity to the carcinogenic activity of non-PNA compounds?

4. Why did the simulated sunlight inhibit the rate of appearance of tumors? Enhancement was expected because of the known carcinogenicity of UV light to mouse skin, although a few scattered reports in the literature indicate that inhibition has been observed under certain schedules of illumination and application of a PNA carcinogen. Is the inhibition due to photodestruction of carcinogens or to modification of the skin, lessening its responsiveness, or to an actual cytotoxic effect on precancerous cells? It is interesting that this response was very pronounced in the Type I pitch and B(a)P groups of CD-1 mice.

5. Why did the CD-1 mice respond more rapidly than the C3H/HeJ to the positive control carcinogen, B(a)P, when their response to the complex mixtures was slower?

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TABLE 1

Test Materials and Associated Temperature Specifications

Material	Synonyms	ASTM Softening Point (°C)	Suggested Field Application Temperature (°C)	Suggested Kettle Temperature (°C)
Type I Asphalt	dead level asphalt	57-66	177-204	204-232
Type III Asphalt	steep asphalt	85-96	218-246	246-273
Type I Coal tar pitch	regular roofing coal tar bitumen; pitch Type A	52-60	177-204	204-232
Type III Coal tar pitch	"no burn" or "low burn" roofing coal tar bitumen (pitch)	56-64	177-204	204-232

TABLE 2

Typical Distribution of Fumes in Collection System

		Impinger					
Material		Ice	Dry Ice/ Isopropanol	Dry Ice/Isopropanol (Second)	Cyclohexane/Acetone Solution	Filter	Total
Type I Asphalt	gm	32	25	3	1	0.2	61.2
(232°C)	%	52	41	5	1.6	0.3	
Type III Asphalt	gm	138	53	17	3	0.1	211.1
(316°C)	%	65	25	8	1.4	0.05	
Type I Pitch	gm	410	86	32	5	<0.1	533
(232°C)	%	77	16	6	0.9	<0.02	
Type III Pitch	gm	720	28	2	1	<0.1	751
(316°C)	%	96	3.7	0.3	0.1	<0.01	

TABLE 3

Mass Emission Rate

Material	Temperature (°C)	Mean Emission Rate (g/hr)	Std. Deviation
Type I Asphalt	232	2.1	1.2
Type I Asphalt	316	33	16
Type III Asphalt	232	3.1	2.6
Type III Asphalt	316	27	13
Type I Pitch	232	38	10
Type I Pitch	316	79[1]	--
Type III Pitch	232	17	12
Type III Pitch	316	115	--

[1] Calculated on the basis of one generation.

TABLE 4

Concentration (µg/mL) of PAHs in Skin-Painting Solutions -- Summary (Asphalts)

	Analytical Ion	Type I 232°C A	Type I 316°C B	Type III 232°C C	Type III 316°C D
Naphthalene	128	22	4.4	17	49
Fluorene	166	36	22	39	28
Carbazole	167	20	1.4	6.3	--
Anthracene/Phenanthrene	178	180	53	300	69
Fluoranthene	202	86	10	97	7.3
Pyrene	202	70	9.0	63	7.7
Benz(a)anthracene	228	11	10	7.6	5.7
Chrysene/Triphenylene	228	25	19	13	14
Benzofluoranthenes	252	1.8	4.0	5.2	--
Benzo(e)pyrene	252	5.5	8.3	3.6	1.4
Benzo(a)pyrene	252	2.2	1.9	2.9	--
Indeno pyrene	276	2.7	3.1	2.2	--
Benzo perylene	276	0.8	1.5	0.8	--
Dibenzanthracenes	278	1.6	--	1.8	--
Coronene	300	--	--	--	--
Dibenzopyrenes	302	--	--	--	--

TABLE 5

Concentration (μg/mL) of PAHs in Skin-Painting Solutions (Coal Tar Pitch)

	Analytical Ion	Type I		Type III	
		232°C E	316°C F	232°C G	316°C H
Naphthalene	128	>1800	1770	288	>620
Fluorene	166	X	740	X	X
Carbazole	167	1980	1450	540	1400
Anthracene/Phenanthrene	178	>960	2960	>2580	>5200
Fluoranthene	202	>2940	2350	>960	>2800
Pyrene	202	>2070	1790	>720	>2300
Benz(a)anthracene	228	570	330	330	800
Chrysene/Triphenylene	228	460	300	290	710
Benzofluoranthenes	252	230	230	250	250
Benzo(e)pyrene	252	42	51	45	46
Benzo(a)pyrene	252	96	85	102	90
Indeno pyrene	276	33	1.7	11	6.8
Benzo perylene	276	28	2	7.2	0.7
Dibenzanthracenes	278	12	-	4.1	-
Coronene	300	-	-	-	-
Dibenzopyrenes	302	-	-	-	-

TABLE 6

Test Solution Composition

Material	Test Code	Temperature °C	Final Concentration of Skin Painting Solutions (mg/ml)
Type I Asphalt	A	232	500
Type I Apshalt	B	316	500
Type III Asphalt	C	232	500
Type III Asphalt	D	316	500
Type I Pitch	E	232	78
Type I Pitch	F	316	55
Type III Pitch	G	232	84
Type III Pitch	H	316	30

TABLE 7

Effective Total

Surviving Animals - As of First Positive Papilloma

(Numbers in parentheses are weeks on test at first papilloma)

		CD-1		C3H/HeJ	
MATERIAL		SOLAR	NON-SOLAR	SOLAR	NON-SOLAR
		No.	No.	No.	No.
A - Asphalt I	232°	17 (56)	47 (24)	48 (33)	49 (30)
B - " "	316°	38 (50)	48 (24)	47 (18)	47 (23)
C - Asphalt III	232°	50 (17)	48 (31)	42 (34)	47 (21)
D - " "	316°	45 (41)	46 (41)	48 (16)	45 (24)
E - Pitch I	232°	44 (31)	47 (22)	45 (37)	46 (33)
F - " "	316°	45 (35)	45 (31)	44 (37)	46 (26)
G - Pitch III	232°	41 (40)	50 (14)	47 (17)	40 (34)
H - " "	316°	48 (29)	48 (28)	42 (39)	45 (28)
Solvent		- - - -	- - - -	43 (40)	- - - -
Solvent, cage			49 (41)		- - - -
B(a)P		49 (28)	48 (28)	44 (40)	42 (38)
Combination (D, F)		44 (21)	45 (30)	44 (21)	48 (25)
None		- - - -		21 (80)	

TABLE 8

Summary of Gross Pathology

CD-1 Mice

	No. of Animals		Liver Enlarged	Liver Pale	Liver O.G.*	Spleen Enlarged	Spleen Mottled	Lung O.G.*
Asphalts	400	No.	68[s]	191[+]	28	88[s+]	50	42
		%	17.0	47.8	7.0	22.0	12.5	10.5
Pitches	400	No.	61[s]	180	17	114[s]	71[s]	42
		%	15.2	45.0	4.2	28.5	17.8	10.5
Combination	100	No.	13[s]	40	5	27[s]	16	13
		%	13.0	40.0	5.0	27.0	16.0	13.0
Benzo(a)-pyrene	100	No.	15[s]	36	4	35[s]	20[s]	12
		%	15.0	36.0	4.0	35.0	20.0	12.0
Controls	200	No.	12	85	12	30	19	21
		%	6.0	42.5	6.0	15.0	9.5	10.5

C3H/HeJ Mice

	No. of Animals		Liver Enlarged	Liver Pale	Liver O.G.*	Spleen Enlarged	Spleen Mottled	Lung O.G.*
Asphalts	400	No.	44[s]	187[s]	122	201[s]	239[s]	102[s]
		%	11.0	46.8	30.5	50.2	59.8	25.5
Pitches	400	No.	53[s]	244[s+]	75[s+]	204[s]	272[s+]	120[s+]
		%	13.2	61.0	18.8	51.0	68.0	30.0
Combination	100	No.	5	52[s]	18[s+]	45[s]	64[s]	34[s]
		%	5.0	52.0	18.0	45.0	64.0	34.0
Benzo(a)-pyrene	100	No.	7	45[s]	30	46[s]	52[s]	17
		%	7.0	45.0	30.0	46.0	52.0	17.0
Controls	200	No.	11	58	61	6	8	23
		%	5.5	29.0	30.5	3.0	4.0	11.5

* O.G. = "outgrowth", or presumptive tumor.

[s] significant by Chi-square at $p \leq 0.05$ as compared to control groups

[+] significant by Chi-square at $p \leq 0.05$ as compared to BaP group

TABLE 9

Tumor Incidence

CD-1 Mice

				GROSS OBSERVATIONS[a]				FINAL HISTOPATHOLOGY							
								TUMOR-BEARING ANIMALS			TUMORS[d]				
EXPT. NO.	TEST MATERIAL		SOLAR	TBA	CBA	TOTAL TUMORS	ET[b]	TOTAL	BENIGN[c]	MALIGNANT[d]	PAP	KA	CA	FS	OTHER
1	A - Type I Asphalt	232°	-	7	1	14	47	8	6(4)+2	0	12				
3	" - "	"	+	4	0	5	17	4	2(1)+2	0	3				
5	B - "	316°	+	5	0	8	38	7	3 +4	0	3	0	0		
7	" - "	"	-	21	2	37	48	21	13(4)+7	1	18	0	0	1	
9	C - Type III Asphalt	232°	-	12	2	20	48	14	9(1)+4	1	11	1	1		
11	" - "	"	+	10	0	14	50	11	5 +4	2	5	0	1	1	
13	D - "	316°	+	4	0	6	45	6	4(1)+1	1	5	0	1		
15	" - "	"	-	17	1	27	46	20	13(3)+4	3(1)	17	0	1	2	
17	E - Type I Pitch	232°	-	36	10	102	47	38	22(10)+7	9(5)	39	7	9	1	2
19	" - "	"	+	27	1	52	44	29	20(11)+8	1(1)	31	5	1		
21	F - "	316°	+	19	2	34	45	23	17(7)+4	2(2)	24	0	1	1	2
23	" - "	"	-	36	11	100	45	38	27(13)+8	3(2)	49	3	2	1	
25	G - Type III Pitch	232°	-	33	9	83	50	37	26(10)+7	4(2)	42	1	4		
27	" "	"	+	20	2	45	41	23	20(8)+2	1	29	1	1	0	3
29	H - "	316°	+	27	2	43	48	28	19(3)+9	0	21	1	0	0	1
31	" - "	"	-	38	7	99	48	41	28(11)+10	5(2)	40	3	5	0	3
33	I - Solvent		-	0											
35	" - "		+	0											
37	M - " (cage)		-	1		1	48	1	1		1				
39	J - Benzo(a)pyrene		-	38	17	101	48	40	24(8)+5	11(6)	43	3	10	3	
41	" - "		+	12	3	25	49	13	9(5)+1	3	11	4	1	2	
43	K - Combination (D,F)		-	30	7	62	45	33	22(9)+8	3(1)	36	3	3		
45	" - "		+	11	2	11	44	15	9(1)+5	1	10	0	0	1	
47	L - Untreated		+	0											

TABLE 9 (continued)

Tumor Incidence

C3H/HeJ Mice

EXPT. NO.	TEST MATERIAL		SOLAR	GROSS OBSERVATIONS[a]				FINAL HISTOPATHOLOGY							
								TUMOR-BEARING ANIMALS			TUMORS[d]				
				TBA	CBA	TOTAL TUMORS	ET[b]	TOTAL	BENIGN[c]	MALIGNANT[d]	PAP	KA	CA	FS	OTHER
2	A - Type I Asphalt	232°	-	47	20	118	49	47	24(10)+1	22(11)	34	10	25	4	3
4	" - "	"	+	43	17	79	48	43	14(8)+2	27(9)	22	10	25	5	0
6	B - "	316°	+	44	24	118	47	44	18(8)	26(8)	36	6	26	4	1
8	B - "	"	-	45	27	128	47	45	13(8)+1	31(13)	27	7	31	9	4
10	C - Type III Asphalt	232°	-	42	19	94	47	42	15(7)+2	25(10)	32	3	19	9	3
12	" - "	"	+	33	18	57	42	34	11(7)+3	20(7)	14	8	19	2	1
14	D - "	316°	+	36	22	80	48	38	20(10)	18(10)	34	7	20	2	2
16	" - "	"	-	40	28	126	45	40	12(8)	28(19)	24	6	36	9	7
18	E - Type I Pitch	232°	-	42	38	122	46	42	11(6)	31(20)	34	6	38	2	3
20	" - "	"	+	45	32	121	45	45	12(9)	33(18)	41	5	39	2	8
22	F - "	316°	+	42	28	94	44	43	13(10)	30(10)	28	7	33	1	2
24	" - "	"	-	45	42	127	46	45	11(10)	34(21)	43	8	40	2	3
26	G - Type III Pitch	232°	-	38	30	115	42	39	12(4)	27(18)	27	8	29	2	5
28	" - "	"	+	43	28	114	47	44	12(9)	32(20)	39	8	39	4	5
30	H "	316°	+	38	21	77	42	40	15(11)+1	24(13)	28	9	24	4	8
32	" - "	"	-	44	37	117	45	45	14(6)	31(21)	25	7	39	7	10
34	I - Solvent		-	0											
36	" - "		+	1	1	2	43	1	1(1)	0	2				
38	M - " (cage)		-	0											
40	J - Benzo(a)pyrene		-	38	24	60	42	38	11(3)	27(13)	12	8	29	2	2
42	" - "		+	34	21	68	45	35	7(3)+1	27(4)	11	5	22	5	0
44	K - Combination (D,F)		-	42	30	161	48	44	14(11)	30(15)	40	7	35	1	8
46	" - "		+	41	23	114	44	41	11(4)	30(17)	25	6	34	4	7
48	L - Untreated		+	1	1		21	1	0	1	0	0	1	0	0

a TBA = Tumor-bearing animals; CBA = Animals bearing one (or more) presumptive carcinomas.

b Effective total, number of animals alive at time of first papilloma.

c Number in parentheses is number of animals with ≥ 2 benign tumors. "+" indicates regressed gross positives

d Number in parentheses is number of animals with at least one malignant tumor plus one or more other tumors, whether malignant, or benign

e Total will not necessarily equal total grossly observed, due to fusion and regression. Tumor types: PAP = papilloma (benign), CA = squamous cell carcinoma (malignant), FS = fibrosarcoma (malignant), KA = keroto-acanthoma (benign), OTHER includes fibroma and unclassified benign epithelioma.

TABLE 10

Mean Time to Tumor
(months ± SD)

Test Material	Solar	CD-1			C3H/HeJ		
		$\bar{X}$	SD	Δ_s*	$\bar{X}$	SD	Δ_s*
Asphalts							
Type I - 232°C (A)	-	12.2	3.7		11.7	2.6	
	+	15.0	2.4	2.8	12.2	2.4	0.5
316°C (B)	-	12.2	3.5		9.2	2.1	
	+	14.1	2.8	1.9	10.0	2.1	0.8
Type III - 232°C (C)	-	12.0	3.0		10.7	2.9	
	+	13.4	4.3	1.4	11.7	1.8	1.0
316°C (D)	-	12.8	2.4		9.4	1.9	
	+	12.2	3.2	-0.6	10.2	2.7	0.8
Coal Tar Pitches							
Type I - 232°C (E)	-	9.7	3.7		9.5	1.2	
	+	12.1	3.0	2.4	10.0	1.1	0.5
316°C (F)	-	10.2	2.1		9.0	1.0	
	+	13.7	2.8	3.5	10.1	1.6	1.1
Type III - 232°C (G)	-	10.7	2.3		8.9	0.9	
	+	12.2	1.5	1.5	9.3	1.5	0.4
316°C (H)	-	10.8	2.8		9.0	1.3	
	+	12.4	2.2	1.6	11.2	1.7	2.2
Combination							
	-	11.4	2.6		8.9	1.5	
	+	13.0	3.6	1.6	10.0	2.1	1.1
Benzo(a)pyrene							
	-	11.4	2.9		13.0	2.4	
	+	13.2	3.2	1.8	14.7	2.4	1.7

---

* Δ_s = Increment due to effect of simulated sunlight.

TABLE 11

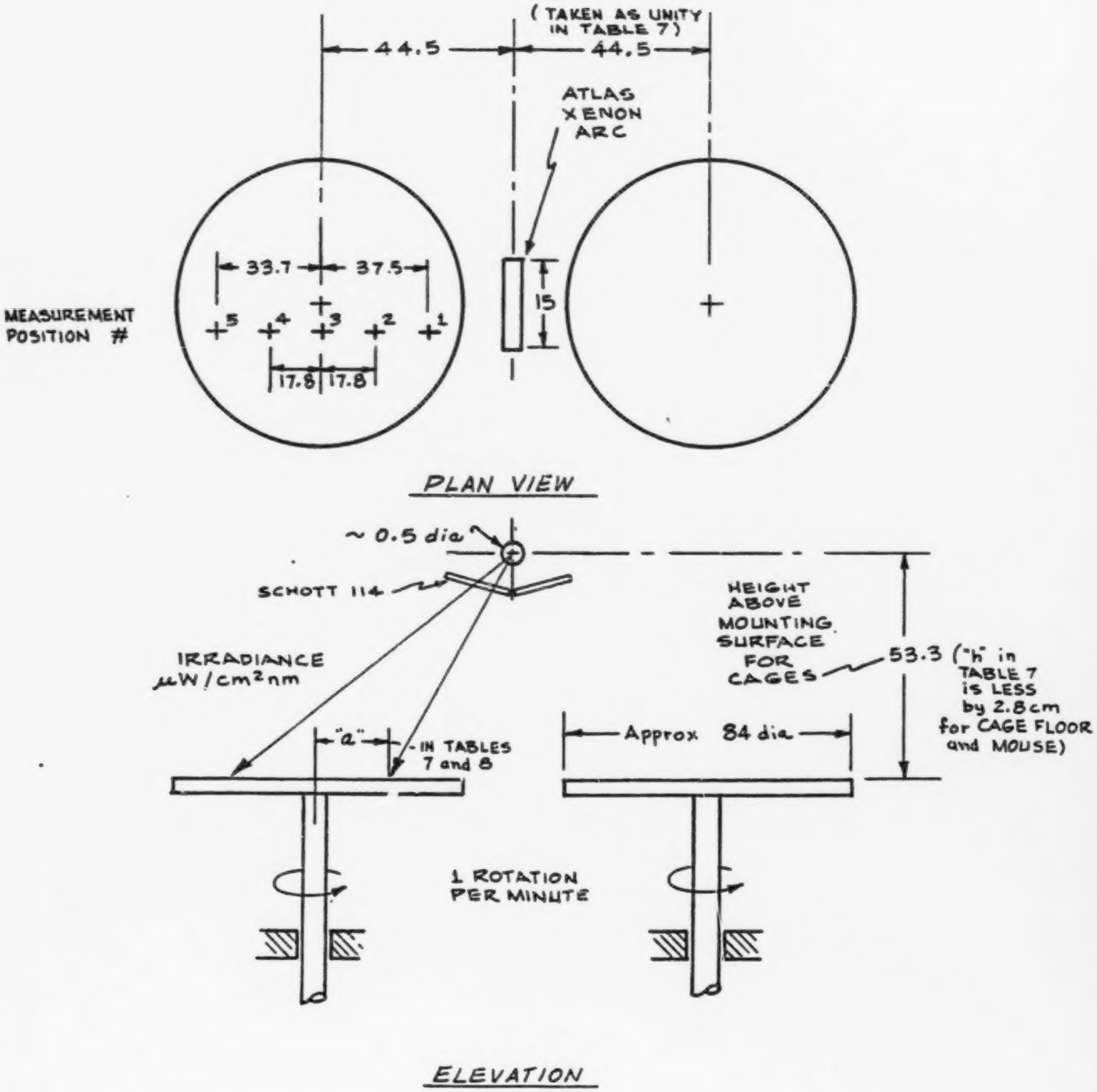
Total Dose of Test Material Applied up to Time of 50% Tumor Incidence (C3H Mice)

Preparation	Time to 50% T.I.* (months)	No. of Applications**	Amount of Material per application			Total Dose Applied***		
			Total Solids mg	PAH µg	B(a)P µg	Total Solids mg	PAH mg	B(a)P µg
Asphalts								
A Type I - 232°	12	104	25	23.2	0.11	2600	2.4	11.4
B Type I - 316°	9.3	81	25	7.4	0.095	2025	0.60	7.7
C Type III - 232°	11	96	25	28.0	0.145	2400	2.7	13.9
D Type III - 316°	9.5	83	25	9.1	<0.025	2075	0.76	<2.1
Coal Tar Pitches								
E Type I - 232°	9.7	84	3.9	>560	4.8	328	>47.0	403
F Type I - 316°	9	78	2.75	603	4.25	214	47.0	332
G Type III - 232°	9	78	4.2	>306	5.1	328	>23.9	398
H Type III - 316°	8.8	77	1.5	>711	4.5	116	54.7	346
B(a)P	13.7	119	-	5.0	5.0	-	0.6	595

* = Tumor Incidence

** = Months x 8.7 (average no. of applications per month at 104/yr.)

*** = No. of applications x amount per application


(TAKEN AS UNITY IN TABLE 7)
44.5
44.5
ATLAS XENON ARC
33.7
37.5
15
MEASUREMENT POSITION #
5
4
3
2
1
17.8
17.8
PLAN VIEW
~ 0.5 dia
SCHOTT 114
IRRADIANCE μW/cm2nm
HEIGHT ABOVE MOUNTING SURFACE FOR CAGES
53.3 ("h" in TABLE 7 is LESS by 2.8cm for CAGE FLOOR and MOUSE)
"a"
IN TABLES 7 and 8
Approx 84 dia
1 ROTATION PER MINUTE
ELEVATION

All Dimensions in cm

Figure 1

Turntables and Xenon Arc Geometry

Figure 2

TUMOR INCIDENCE (%)
(Pooled Data)

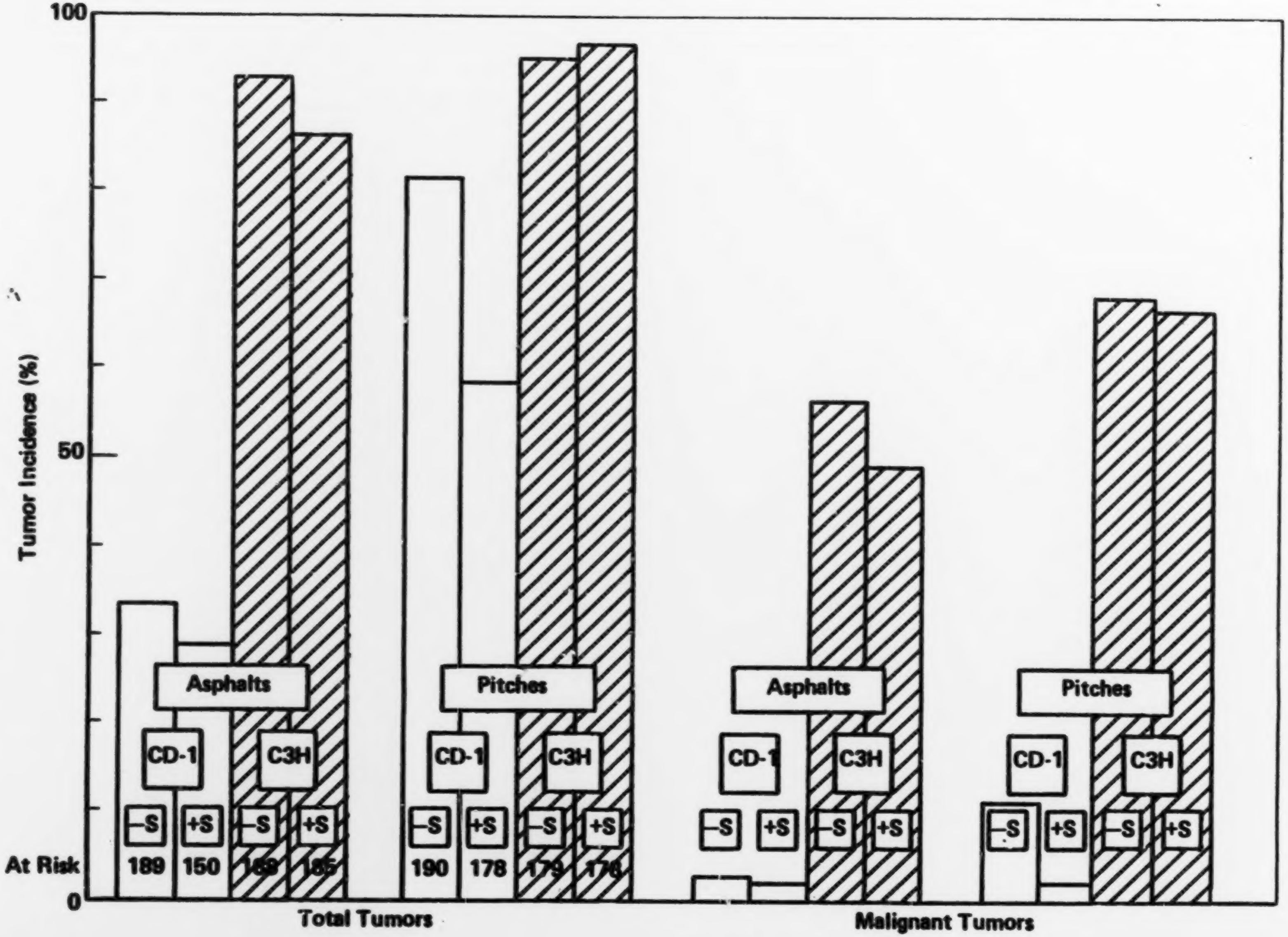
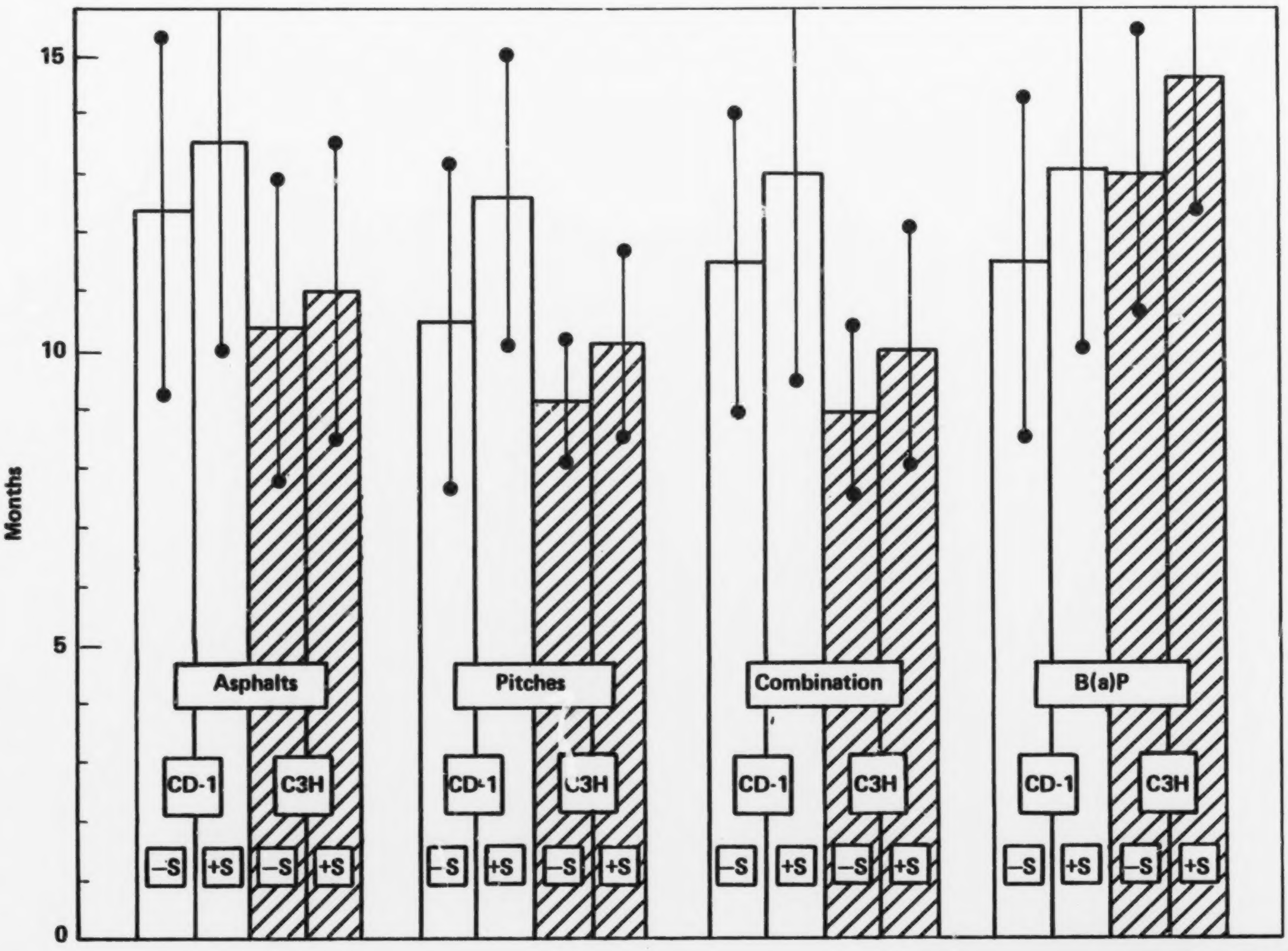


Figure 3

MEAN TIME TO TUMOR

(Pooled Data)



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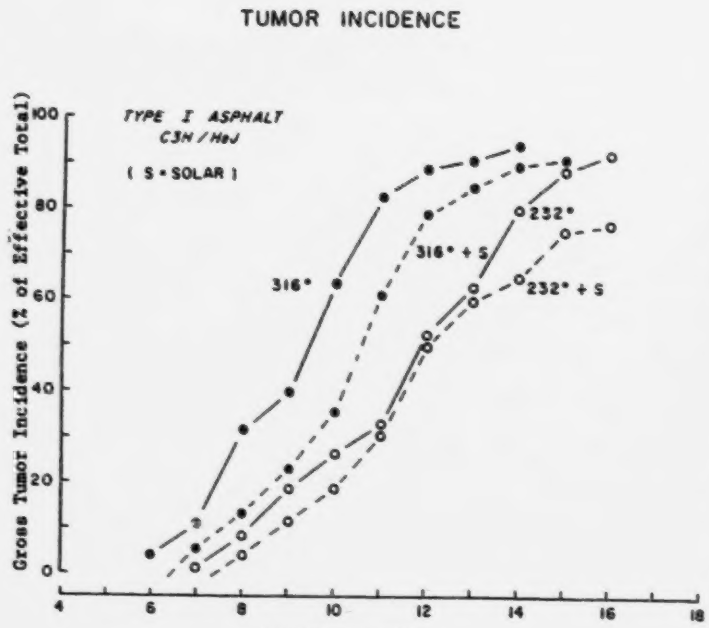


FIGURE 4

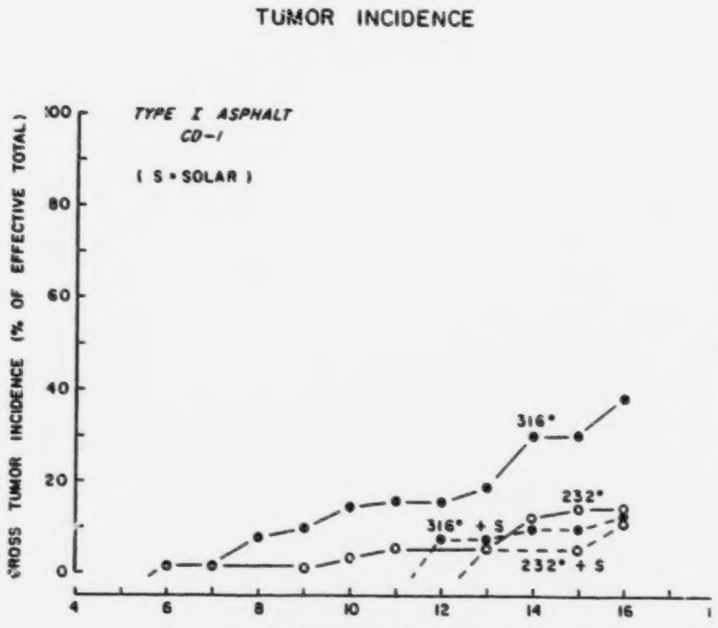
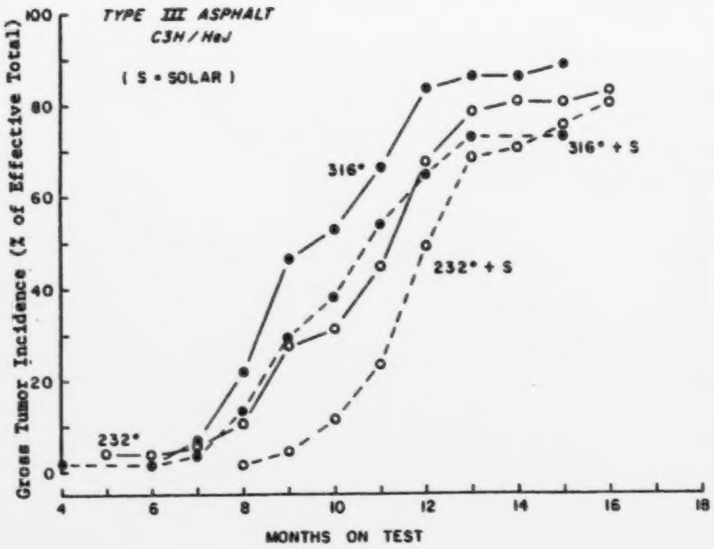


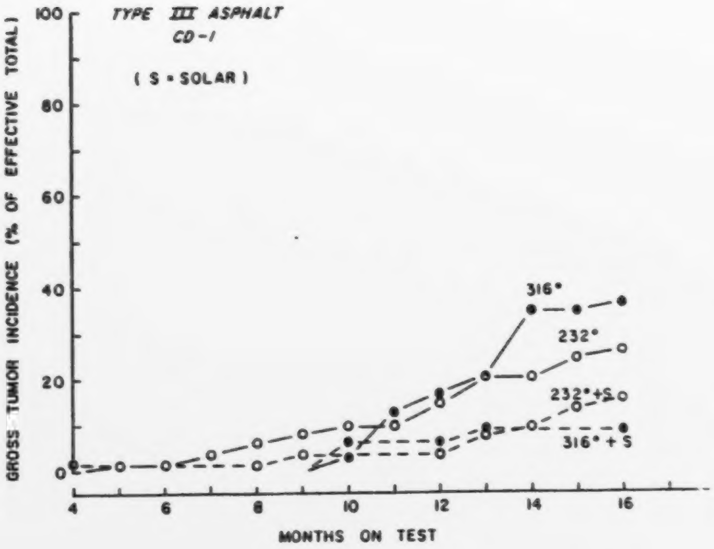
FIGURE 5

533 **BEST DOCUMENT AVAILABLE**



TUMOR INCIDENCE

FIGURE 6



TUMOR INCIDENCE

FIGURE 7

534

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TUMOR INCIDENCE

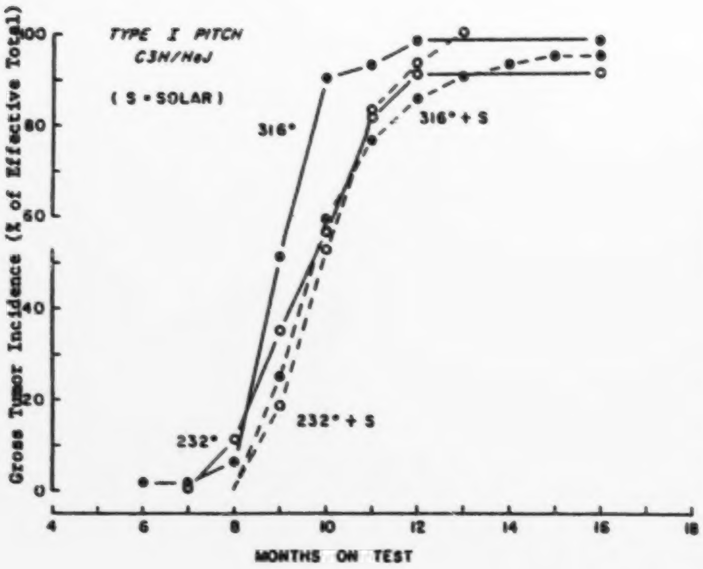

FIGURE 8

TUMOR INCIDENCE

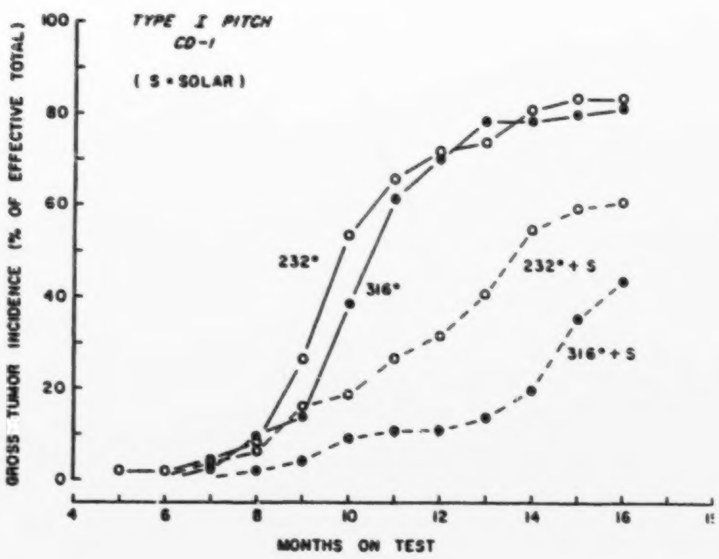

FIGURE 9

535

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TUMOR INCIDENCE

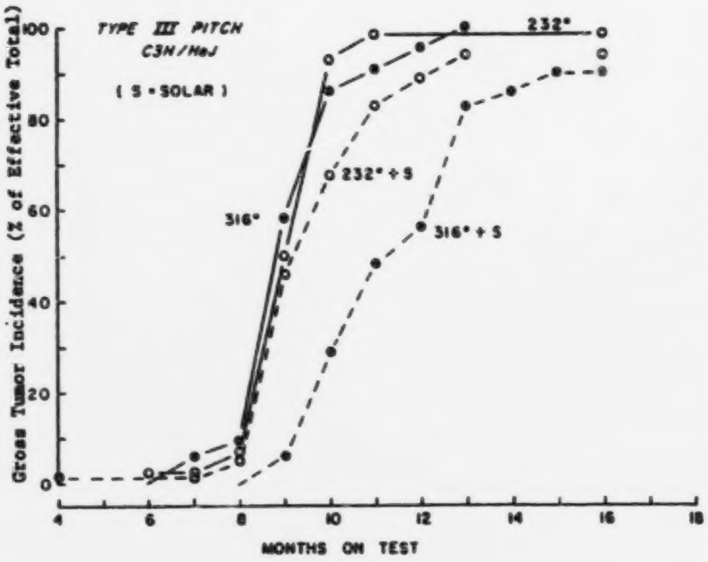

FIGURE 10

TUMOR INCIDENCE

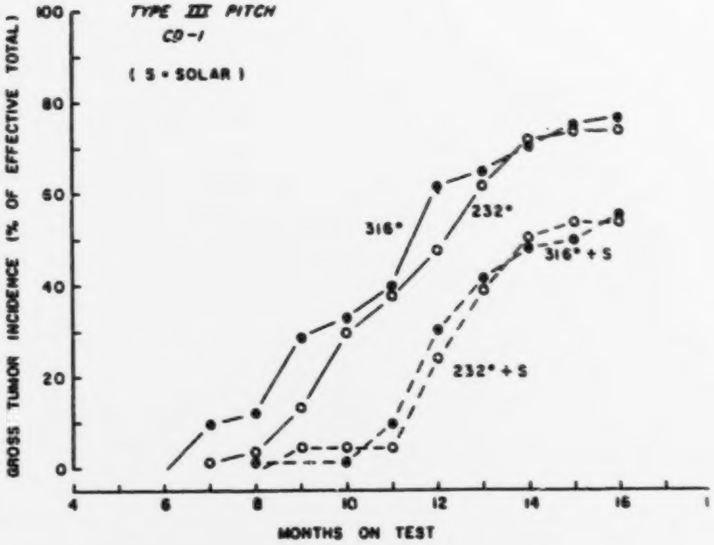

FIGURE 11

536

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TUMOR INCIDENCE

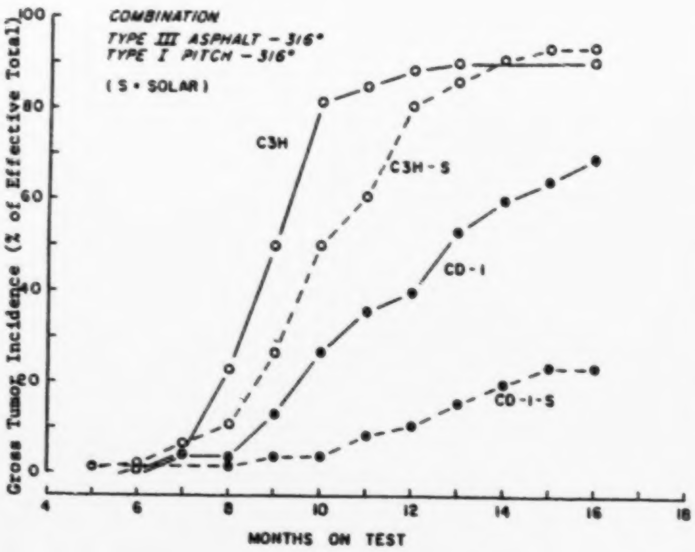

FIGURE 12

TUMOR INCIDENCE

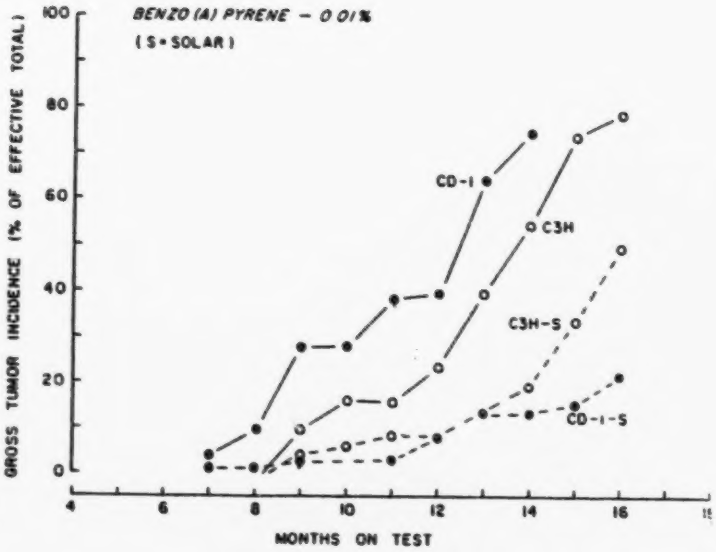

FIGURE 13

DR. GREGORY: I am looking for an explanation for the inhibitor effect of the UV. Did I understand that when you used the UV that you had increased ventilation over the non-UV, or was the ventilation the same?

DR. NIEMEIER: The ventilation was always the same. The animals were treated, skin-painted, put in their cages that fit on the turntables and then all animals, except for those cage controls, were placed in that ventilated box -- the UV, or sun exposed on the top and the non-exposed on the bottom.

DR. GREGORY: What is the explanation?

DR. NIEMEIER: The explanation for the difference of the sunlight versus non-solar effects? There could be many possibilities: one, that the carcinogenic chemicals are photooxidized; it could be that the UV light is toxic to precancerous cells or the transformed cells. I don't know at this point because there are a number of possibilities. And we will be looking at this in future studies.

PROCEEDINGS OF THE
SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

Friday Morning, September 11

DISCUSSION GROUP

GROUP A - EPIDEMIOLOGY

Discussion Leader:
Dr. Wayne Galbraith
Environmental Protection Agency

DR. GALBRAITH: I am not an epidemiologist; therefore, I would appreciate assistance from the epidemiologists present. It would be appropriate and desirable to make comments regarding the quality of the studies presented this morning and possible future directions.

I would like to make the suggestion that in the future, when presenting studies, for which we have incomplete data or for those recently initiated, more time be devoted to the justification for study initiation. It would be of interest to know a little bit more about the justification for the wood preservative study. Is there a documented health problem in this industry? We are dealing with a relatively small population. We know that the chemicals are toxic. Are the safety measures currently being taken in the plant such that there is no problem, or is there a documented problem? Is there a justification to spend resources on experimental work when a problem doesn't currently exist? Or should we wait until there is an indication of a problem?

The floor is open for comments.

DR. AUSTIN: I could just mention two problems that were noted with wood preservatives in California, and they are two quite unrelated problems. There are, in northern California, a number of very, very small companies, putting creosote on poles, primarily telephone poles. The Hazard Evaluation unit in the State Health Department got complaints from workers in one of these plants that several of their members had developed diseases, including cancer and some skin problems. As a result they took a sample of the coating materials and found it to be one of the most highly mutagenic by the Ames test that they had tested among biologic samples that they had picked up. So, at least on theoretic grounds, these workers that are working in the small plants, (only 10 to 15 people in each plant) would be coming in contact with some highly mutagenic materials.

The controls that were shown in today's examples were not anywhere as poor as the controls that were found in some of these small companies.

Secondly, I wanted to mention that arsenically treated wood piers, where the people are fishing from the pier and are laying fish on the pier, is a means of picking up pretty high levels of arsenic from the pier. In one instance, this problem was handled by spraying the wood on top of the pier to cover up the arsenic.

So without having to wait for bodies in the streets, we should recognize that little bits of information came from such situations which suggest that maybe we should take a more serious look at them.

DR. GALBRAITH: Thank you. Are there any other comments?

DR. BAYLISS: I am Dave Bayliss with EPA. We are in the business of doing risk assessments in the carcinogen assessment group. And one of the problems with the epidemiology studies is that although we have risks given on whether or not someone is going to develop cancer or not, most of these studies don't give us any exposure data. You know, we would like to make a plea in future "epi" studies for people to provide us with that data, not just length of employment, which is a surrogate, but we would actually like to have the data itself. Thank you.

DR. GALBRAITH: There was a very interesting paper on mouse skin-painting studies. Could someone comment on the use of the mouse model versus other animal models for skin cancer.

(No response)

I don't know how the studies were selected in the NIOSH/NCI collaborative agreement. It would be of interest to learn how these studies were chosen for funding by that mechanism. Most of the studies presented were from NIOSH/NCI mechanism.

DR. BURTON: The NIOSH program had been already operating for several years before our Branch was brought into it. The NIOSH program had been given four million dollars a year by NCI to investigate matters of joint interest to NCI, as well as to NIOSH. About a year ago it was decided to make this more collaborative than a grant of money to NIOSH.

DR. GALBRAITH: I believe a message is being passed to us, requesting that we adjourn. Dr. Burton, is there a criterion for study selection in the NIOSH/NCI mechanism?

DR. BURTON: The studies went to an evaluation committee, comprising administrative-scientific personnel from both sides. Usually the mechanism was to prepare a project plan for each study giving a very good summary of what was to be done. Then each agency individually evaluated the project plan after which, in a joint session, face to face, the projects were discussed, and it was decided which ones should be funded or not.

DR. GALBRAITH: Thank you.

PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

Friday Morning, September 11

DISCUSSION GROUP

GROUP B - Experimental Methodology/Models

Discussion Leader:
Dr. Elizabeth Weisburger
National Cancer Institute

DR. WEISBURGER: This session is supposed to be on the experimental methodology and the models that are used in all these projects which we have heard about this morning. Of course, in this type of work, improvement in animal models is always an important point, because it does speed up obtaining results, and cuts down the expense and time involved in the experiment.

So I throw this session open for any comments people may have. Please do use this microphone over here, if you have anything to say, and do state your name before making your comment.

So, does anyone wish to bring up any bright ideas in the area of new models for these types of experiments, improvements in methodology?

DR. HORAKOVA: Horakova. I would like to ask Dr. Norman if he did not have trouble using repeated intratracheal instillation in hamsters, because, you know, this is really tricky to do, chronically, every day. I would like to know how much they have taken orally from these applications, or how they did it.

DR. WEISBURGER: You should contact Dr. Saffiotti, who is here at this meeting. There is a little device which more or less holds the mouth open, so that it is really very easy to put the material into the trachea.

DR. HORAKOVA: Yes, maybe, but we always found something is in the stomach.

DR. WEISBURGER: Well, there is some little device which seems to facilitate the process, and maybe you should discuss this with Dr. Saffiotti. Dr. Nettesheim, who is now at NIEHS, has also done this repeatedly, and you may, perhaps, contact him.

DR. HEGYELI: Hegyeli, NCI. I am very happy to hear that other species of animals, not just mammals, are used for toxicity studies. Dr. Harshbarger is going to discuss the use of fishes in toxicity at an NIH conference scehduled for December 1981. We considered the question of a variety of appropriate animal models during my work at the Battelle Memorial Institute in 1967. Based on this survey I would suggest that we also consider the use of clams for toxicity testing. Bivalve mollusks filter large amounts of water within a short period of time. They are also able to strongly concentrate toxic chemicals in polluted habitats. Thus they can be used for the analysis and identification of toxic chemicals.

DR. WEISBURGER: Any further comments on additional animal models which might be helpful in these types of experiments? As Dr. Hegyeli has mentioned, Dr. Harshbarger does have various aquatic animals, and you may have seen the plaque outside, or the little poster outside, announcing the conference to be held in December on the use of small fish as models for testing toxic and carcinogenic materials.

And the advantage there is that one small tank will hold several hundred small fish, so that the space requirements are really cut down greatly, and the need for cleaning of cages, and that sort of thing, is also reduced, so that labor costs are much less. And it is a very efficient system.

DR. HEGYELI: I would like to mention that during my work at the U.S. Army Medical Bioengineering Research and Development Laboratory at Fort Detrick, fishes were used on a large scale for safety testing of recycled water.

DR. WEISBURGER: Do we have any other comments on models or methods for facilitating experimental procedures?

Perhaps we should discuss a bit whether anyone has suggestions and improvements on analytical methodology. This goes along with the improvements in the animal models, too, because, after all, all these people are out there analyzing for benzopyrene and chrysene and all the other polycyclic aromatic hydrocarbons, and other types of compounds, for example, nitrosamines or aromatic amines -- all known carcinogens, or known to contain carcinogens.

And improvements in methodology would certainly speed up the process in these types of experiments. Does anyone have comments or questions on any aspect, or any part of that operation?

DR. SIVAK: Sivak, Arthur D. Little. I'm not sure that the necessity is improvement in methodology in chemical analysis. I think we have become very expert in how to detect parts per trillion of almost anything that we care to measure, and I think the presentation that was just made by Dr. Niemeier on the work that was done at Arthur D. Little is an example of the kind of thinking that has to go, not in how we analyze better, but how we make use of the data once we get it back. Because we deal with complex substances like polycyclic aromatics, or extracts of Ohio River water, or anything else where we're dealing with multiple materials, to try to segregate out what piece of a biological event that we're measuring is related to the kind of chemistry that we're seeing, I think is going to need a lot of thought that neither improvements in methodology and chemical analysis or in bioassay is going to give us.

It's a new, integrative kind of thing that I don't have any answer for. But I think that some funds and efforts need to be expended on how do we analyze the biological effects of complex materials, using the best chemistry that we have.

DR. WEISBURGER: Well, in addition to your philosophical question, I think we need more work on the interaction of mixtures and their effects. Too many of the bioassay programs thus far are inclined toward testing one compound, and yet that's not realistic, because out in the world, we're exposed to many sorts of things every day.

Does anyone have any comments on that sort of approach?

DR. HEGYELI: In the field of occupational cancer prevention, as Dr. Sivak mentioned, the worker is seldom exposed to chemically pure substances, but the toxicity tests are performed on pure substances. What we need to do in the future is to test the mixtures, crude intermediates and crude final products the workers are exposed to for toxicity and carcinogenicity.

DR. WEISBURGER: Yes. Furthermore, and it's perhaps very good for the human race, we see that these carcinogens, in many cases, seem to counteract the effects of each other, and that was shown in the last paper, where the simulated sunlight, or ultraviolet, seemed to cut down on the response to the polycyclic aromatic hydrocarbons.

So, even though we're exposed to a variety of things, they seem to counteract, in a sense, in many cases. It would be helpful, I think, if we knew which of these influences counteracted each other, and which synergized the effects of each other. And, thus far, we don't have experimental systems to do that sort of thing, or it's so massive a project, I think, we've thrown up our hands in horror and not done anything.

DR. SAFFIOTTI: Saffiotti, NCI. I'd like to follow up on your comment, because there is a critical problem that we have to face, i.e., the evaluation of these interactive effects.

As you know, we have started to do some work in the lab and plan to devote more attention to this problem. The development of a range of relatively short-term techniques has made the approach to these studies much more feasible than when we were only relying on long-term animal studies, which require enormous resources in order to study various combinations of factors.

In some of the short-term systems, obviously, the biological conditions may not quite correspond to the complexity of the interactions that occur in vivo; for example, metabolic pathways may occur in vivo that interfere with each other, and may not occur in the in vitro counterparts.

However, the approach we are pursuing is to use a series of biological model systems linking together, in a step-by-step fashion, the simpler in vitro systems to the target organ system terms in animals and in humans. I am particularly referring to epithelial cell culture systems and organ culture systems, related to selected target organs. A battery of such systems can be used to study comparative metabolism in the organs in vivo, then in isolated organ explants, and then in isolated target cells -- such as bronchial cells, or skin cells. In those cells, transformation and mutational events can be measured.

One can thus begin to correlate some of the important biological endpoints and some of the mechanisms, by step-by-step experimentation, with the event in the whole animal and in the human counterpart.

We have observed in the Ames test, which is almost the simplest of these steps, marked effects of mutual inhibition on the part of several polycyclic hydrocarbons; if they should be also found in the yet unexplored more complex systems, they may indicate that biological effects of mixtures measured through some of these systems may reflect a marked difference from the sum of their components tested individually.

The other aspect of this problem, that refers to some of the interesting data presented earlier this morning, is that of interactions of different types of cofactors, such as the effect of particulates in the respiratory tract, and on the activity of carcinogens.

We don't know yet many of the mechanisms underlying these effects, but through the combined use of all the new model systems, more of this type of work becomes possible. For example, characterization of physical characteristics as parameters that can be used in predicting some of the interactive effects of particulates on organic carcinogens.

So, I think there is a lot of research, which has to be conducted at a fairly basic level of sophisticated methodology, that can really be addressed to problems having an immediate impact on the evaluation of as occupational hazards.

DR. WEISBURGER: Thank you, Dr. Saffiotti.

Do we have any other comments?

DR. FAIRCHILD: I am Edward J. Fairchild, University of Texas. I have nothing to add in terms of difference, but I do want to follow up on this theme that has been presented; prior to going to Houston with the University of Texas, I was with the World Health Organization in Geneva. They were very interested in and were supporting studies concerning the effects of combined exposures.

Primarily in terms of survey of the literature, the WHO found many discrepancies regarding the subject of combined exposures. As example, we often hear much of heat exacerbation of the toxic effects of chemicals; but there is a lot of work to show this is not the case. We saw an example of heat exacerbation this morning, possibly, with light.

Some studies I can think of as examples, like in Bulgaria, they were very interested in the combined effects of heat exposure to women who are working with pesticides in large green house enclosures where they have high heat and high humidity. They were studying these various factors, and found some exacerbation of some of the pesticide effect in these exposed populations, but I believe I recall that the trends they observed could not be duplicated in animal experimentation.

WHO is in the process of sponsoring an international conference on the subject of effects of combined exposures, and has already put out one publication on the subject. It is titled "Health Effects of Combined Exposures in the Work Environment." It is Technical Report Series No. 662, A Report of a WHO Expert Committee, 1981. I thought it might be of interest to the group.

DR. WEISBURGER: Thank you very much. I'm sure that everyone is glad to know that this was occurring.

PROCEEDINGS OF THE
SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

Friday Morning, September 11

METHODOLOGY, EXPERIMENTAL, AND MODELS SESSION - continued

Session Chairperson:
Dr. Carl R. Morris
Environmental Protection Agency

DR. MORRIS: This session deals with methodology and experimental models. We have done a little switching around and we would like to have the PAHO study first, if we may. Then we will go on to Ted Jorgenson. He is going to substitute for the first talk, presently scheduled for 11 o'clock. So we will do a little switching. Bear with me and I will make the announcements as we go along which papers are in what order.

The first talk will be the Possible Teratogenic and Carcinogenic Effects of Pesticides on Human Health. Substituting for Jane Keller will be Dr. Torloni. Dr. Torloni.

PROCEEDINGS OF THE
SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Possible Teratogenic and Carcinogenic Effects of Pesticides on Human Health

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TERATOGENIC AND CARCINOGENIC EFFECT OF PESTICIDES ON HUMAN HEALTH

E.P.A. Contract No. 68-01-6251

Semi-annual Report # 1

1 October 1980 - 31 March 1981

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POSSIBLE TERATOGENIC AND CARCINOGENIC EFFECTS OF PESTICIDES ON HUMAN HEALTH*

INTRODUCTION

On 30 September 1980, the Environmental Protection Agency (EPA) awarded a four-year contract (No. 68-01-6251) to the Pan American Health Organization in support of two studies--one on the possible teratogenic effects of pesticide exposure in humans and the other on the possible carcinogenic effects of pesticide exposure in humans, both to be conducted in Colombia, South America. The contract funds, $1,031,502 for a four-year period, are provided through an inter-Agency agreement between the National Cancer Institute and the EPA.

The studies will be developed with the collaboration of the International Agency on Research on Cancer (IARC) in Lyon, France; the National Institute of Health of Colombia in Bogota and the University of Miami, in Florida, U.S.A., the Pan American Health Organization, the U.S. Environmental Protection Agency and the National Cancer Institute.

This report is submitted in compliance with Article III of the above mentioned contract. It covers the period 1 October 1980-31 March 1981 and deals mainly with the teratogenicity study, since preparations for the second study on the carcinogenic effects is scheduled to begin only during the second semester.

I. TECHNICAL PROGRESS REPORT

The main technical activities completed during the reporting period are:

(a) Census of workers in floriculture, formulating plants and sanitary campaigns in Colombia, South America.

(b) Preparation of the final version of the questionnaires for the teratogenesis study: (1) questionnaire for the floriculture workers and (2) questionnaire for the cases and controls.

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* Contract between PAHO and EPA No. 68-01-6251. First semi-annual report covering the period 1 October 1980-81 March 1981.

(c) Site visit of the National Institute of Health in Bogota by the Principal Investigators from the University of Miami, and the International Agency for Research on Cancer.

(d) Meeting of Principal Investigators from the University of Miami, the International Agency on Research, the Environmental Protection Agency and the Pan American Health Organization.

(e) Recruitment and training of interviewers.

The above represents an on-schedule completion of all activities anticipated in the original plan of work with the exception of the interviewing of floriculture workers to identify parents of children with congenital malformations which was planned to begin in February, but could only be initiated in March 1981. The main reason for this delay was the lack of transportation for the interviewers.

Below is a summary report of these activities:

1. Census

(a) Floriculture Workers

The census was undertaken with the cooperation of the Colombian Flowers Exporters' Association (ASOCOLFLORES) and included 44 enterprises in the savanna of Bogota. Thirty nine of these enterprises agreed to collaborate. The census was based on the lists provided by the participating enterprises and identified 11,000 workers, of which 940 are office staff. However, since the completion of the census last December, the number of exposed workers has been revised to 13,000 due to an increase in the number of enterprises which now amounts to 55. The growth in the enterprises reflects splits of large ones as well as new companies. Of the exposed workers, 86% or 11,180 people are between 15 and 40 years of age; of these 11,180 people 64% or 7,155 are female and 36% or 4,025 are male. The average number of workers per enterprise is 236. The average number of workers hired in each enterprise yearly is 92. The average number of workers quitting their jobs per year is 89 per enterprise. The average time on the job of the current workers is 2.7 years. Among the several job classifications are sorters, thinners, pruners, packers, cutters, mixers, fumigators, rubber band applicators (to synchronize blooms), and packers. Some of these jobs are sex specific; cutters and fumigators are male dominated while thinners and pruners are primarily females. In some jobs, workers wear protective clothing (e.g. sprayers wear masks and gloves).

A list of the pesticides and the quantities used between 1958 and 1979 is being obtained.

Attachment III contains 6 tables with the frequency distribution by age, sex and duration of employment of workers in floriculture, formulation plants and sanitary campaigns.

As a complement to the above mentioned census of workers, a list of persons formerly employed in formulating plants and sanitary campaigns is being obtained. This list is essential for the second planned part of the project, the retrospective study of cohorts for possible carcinogenic effects of pesticides.

2. Preparation and Testing of Questionnaires

The preliminary questionnaires, one for the study of teratogenesis and the other to evaluate exposure to pesticides, were merged into one, which was administered to a sample of 300 workers employed--100 in floriculture, 100 in formulating plants, and 100 in sanitary campaigns.

3. Visit to the National Institute of Health in Bogota

Drs. Nubia Munoz and John Day from the International Agency for Research on Cancer (IARC) in Lyon, and Drs. Carl D. Pfaffenberger, John E. Davies, and Robert S. Levine from the University of Miami visited with Drs. Enrique Guerrero and Mauricio Restrepo at the National Institute of Health in Bogota, Colombia. Dr. Munoz visited Colombia during the period 25 January-7 February 1981. The remaining investigators' visit took place during 25-31 January 1981.

The purpose of the travel was to review the results of the feasibility study that was conducted during 1 September 1979 to 31 August 1980 under the sponsorship of IARC, the data collected in the census, the proposed questionnaires, the pesticides to be included in the exposure assessment, and the adequacy of the study design as originally proposed.

The conclusions of the discussions were that the floriculture workers as the study population offer many advantages because, among other factors, it is large in number; most workers are at a reproductive age; it includes females and males; abortions and deliveries are performed at hospitals; there is exposure to a wide range of pesticides making internal comparisons possible; of the four pesticides most frequently used, captan is a recognized teratogen; and the companies are willing to cooperate. However, at the same time, the preliminary results also revealed some weaknesses such as: (a) turnover of workers in the floriculture companies is very high: the average number of workers entering each company per year is 92 and the average number of workers leaving each company per year is 89; (b) the average period in each company is 2.7 years and for many companies it is only during the last two to three years that they have started keeping records of all the people they have ever employed. Previous workers will be hard to identify and the inclusion in the study of those few that could be identified could lead to serious bias, and (c) interpretation of blood and urine levels of pesticides and their metabolites can only be taken as a measure of present exposure as they indicate exposure during the previous 24-48 hours, and therefore to infer past exposure from present levels would be misleading.

Over the past 25 years, pesticide use in Colombia has risen rapidly. Between 1955 to 1960, pesticide consumption was reportedly low. A 1977 report, however, indicated that 180 active substances were registered which were used to make 640 commercial products. This totalled to 23,000 tons of active ingredients (64% insecticides, 21% fungicides and 15% herbicides). Forty-eight percent of the products were mixtures and 52% are single preparations. Following the discussions concerning the pesticides involved, the principal investigators including the biochemists from Miami and Bogota decided to limit the study to three major groups (carnations, roses and chrysanthemums) and to concentrate on the 22 pesticides used in excess of 1,000 kg. annually, listed in Attachment II.

Four pesticides, all fungicides of current interest to E.P.A., represent over half of the total usage (weight basis). Approximately 23,550 kg. of captan; 10,760 kg. of mancozeb; 8,670 kg. of sodium metham and 8,490 kg. of propineb are used in Colombian floriculture each year according to data collected in 1979. Captan, which represents about 25% of the total usage, is currently on the E.P.A. Rebuttable Presumption Against Registration (RPAR) priority list (1981) and is a confirmed rodent teratogen. Its major metabolite, tetrahydrophthalimide, is structurally similar to the major metabolite of thalidomide, a known human teratogen. Mancozeb and the other bis - thiocarbamates are presently being given close scrutiny inasmuch as they yield carbon disulfide, a known neurotoxic agent, and most of them yield either the rodent carcinogen, bis-ethylenethiourea (ETU), or one of its analogues. It became evident during the discussions in Bogota that this investigation is indeed both timely and urgently needed in order to better assess possible health effects resulting from exposure to these fungicides and other pesticides during pregnancy.

Among the 22 most commonly used pesticides are some common organophosphorus compounds, a few carbamates and some pesticides which do not fit readily into any broad category (Attachment VI). Because many samples will be collected, the number of individual analytical procedures run in lab will be initially held to eleven. Since it is still maintained that serum analysis for organochlorine pesticides and metabolites will be a useful index of pesticide exposure in general, the following analyses will be made whenever appropriate:

(a) Blood will be analyzed for -
  A. Plasma cholinesterase
  B. RBC cholinesterase
  C. Organochlorine pesticides and metabolites
  D. Intact organophosphorus pesticides

(b) Urine will be analyzed for -
  A. Creatinine
  B. Alkyl phosphates
  C. MCA and DCA from malathion
  D. Carbon disulfide
  E. bis-Ethylenethiourea and its analogues
  F. Tetrahydrophthalimide
  G. a-Naphtol and/or other phenolic compounds

The burden of lab analysis will be shared with the Bogota laboratory which, at first, will run cholinesterase, creatinine and organochlorine determinations until after thorough training in the other procedures.

Not all eleven analyses will be required for all samples. Different pesticides are used on different crops, and background information on each sample will greatly assist the chemists in deciding which analyses should be performed. Environmental assessment by air sampling of the three major crops will also aid in making these decisions.

A final dimension which will be added to the study at no additional cost will be cytogenetic and cytokinetic measurements of the 22 floricultural pesticides using human lymphoid cell line LAZ007. These tests will include the effects of the pesticides on (1) sister chromatid exchange frequency, (2) chromasomal replication, (3) chromasomal structure and (4) cell cycle traverse.

The discussions in Bogota proved to be very useful for all Principal Investigators in the Program. It not only allowed them to became personally acquainted, but also to exchange views, find solution to problems and propose adequate modifications of the study design that should improve the quality of the results obtained.

4. Meeting at the University of Miami

This meeting was held on 20 February 1981 and was attended by Jane Keller, M.P.H., U.S.E.P.A.; Nubia Munoz, M.D. I.A.R.C.; John E. Davies, M.D., Carl B. Pfaffenberger, Ph.D., and Ana Barquet, Ph. D. of the University of Miami; and Jorge Litvak, M.D., PAHO.

The purpose of the meeting was to discuss the findings of the site visit to Bogota and the possible modification of the study design.

The main modifications proposed as a result of the discussions in Bogota concerning the retrospective case-control study using measurements in blood and urine are: (1) to confine the retrospective case-control study to women who are currently employed, or whose husbands are currently employed in the industry, and (2) to design the measurement of pesticides exposure levels and the identification of the pesticides concerned or their metabolites in a prospective approach.

In view of the above, the teratogenesis study, that will only include floriculture workers, would consist of three phases: a prevalence study, a retrospective case-control study, and a prospective study.

The prevalence survey will cover all 13,000 floriculture workers. It began in mid March 1981 by seven professional interviewers who are using the questionnaire, to obtain a full reproductive history of the female workers and the present spouse of the male workers, with background demographic data and preliminary information on their work in floriculture. The congenital malformations identified will be compared with medical records and those still alive will be submitted to clinical examination. The interviews are expected to be completed by mid June 1981.

The data will be processed by the Departamento Administrativo Nacional de Estadistica (DANE) of the Natonal Institute of Health in Bogota. Copies of the magnetic tapes will be sent to IARC to assist in the analysis. Data processing will be done keeping pace with the data as they arrive. A complete data tape should be available at the end of June 1981.

Prevalence rates for different types of malformation will be computed separately for female workers and the wives of male workers by age and birth order. The comparisons to be made are between prevalence rates among floriculturists and the general population, taking account of age and parity, and prevalence rates within the cohort, by whether the women (or her spouse) was employed in floriculture during her pregnancy, and if so, by her occupation as given by the preliminary data.

It is anticipated that the analysis of the prevalence survey should take two-and-a-half to three months.

The retrospective case-control study will obtain information by interview using thje draft questionnaire under Attachment V and by review of company records. For each malformation within the cohort, two control normal pregnancies also from the cohort will be chosen, of the same birth order as the malformation and matched for age of the mother at the time of the pregnancy. Control pregnancies will be chosen at random from all those eligible, a process in which the IARC will assist. The choice of controls should be made by the end of June 1981. The women should have been employed in the floriculture industry throughout both the case and the control pregnancies.

A clinical geneticist will examine all those children with congenital malformations and still alive along with the available medical records. A physical examination of the control children will also be done by Dr. M. Restrepo.

The expected number of pregnancies (20%) from the estimated (7,155) total population of female workers of reproductive age is 1,431 per year and 4,293 for a three-year period. The expected number of children with major congenital malformations is 86 or 2% of 4,293. The estimated number of children with congenital malformations among wives of male workers is 48 over a three-year period (male population of reproductive age = 4,025; pregnancy rate of 20% of 4,025 = 805; 2% malformations of 805 = 16 per year). Thus, the total number of children with major congenital malformations is expected to be 134, if the prevalence is the same as in the general population. Two controls (268) will be selected for each case and they will be matched by age of the parents and by birth order.

The interviewing is planned to start in September 1981 and to last four months. As with the prevalence study, data processing will be performed in the computer center at DANE and a tape provided to the IARC when the data entry is completed. The IARC will assist in the analysis.

The prospective study will be based on pilot studies to guide the analysis of pesticides in blood, urine and air samples, including: (a) background levels of organochlorine and organophosphate pesticides in blood and urine from a small sample of floriculture workers. As mentioned before, the first set of specimens were analyzed by Dr. Pfaffenberger and the results are shown in Attachment IV; (b) background levels of pesticides in air samples. Three types of air samples will be taken: during fumigation, shortly after (approximately one hour) fumigation and then later (approximately three hours) after fumigation. Three types of flowers will be used: carnations, roses and chrysanthemums; and (c) studies on the interlaboratory variability on the chemical analysis of pesticides between the Miami laboratory and the Bogota laboratory.

The case-control study within a prospective cohort will analyze blood and urine specimens collected from all female workers and wives of male workers who become pregnant, as early in the pregnancy as possible. Cholinesterase and creatinine respectively will be tested in all specimens immediately after collection in Bogota. Aliquots of 10-20 mls of blood and urine will be stored at -20 C, and only those specimens belonging to mothers who will eventually give birth to a malformed child, and if possible, of those mothers having abortions, and of control mothers, will be analyzed for pesticides or their metabolites, at the end of a two-year, follow-up period.

In the prospective cohort study, pesticide exposure will be assessed by personal interview, review of company records, measurement of selected pesticides and their metabolites in blood and urine, and measurement of pesticides in air samples. From the total population of 7,155 female workers in reproductive age, the number of pregnancies among female workers in two years is expected to be 2,862 and of children with major congenital malformations 58. From the total population of 4,025 male workers in reproductive age, the numer of pregnancies among wives of male workers in two years is expected to be 1,610 and of children with major congenital malformations 32. Thus, the total expected numer of children with major congenital malformations (58 + 32) is 90. As in the retrospective case-control study, two controls per case will be selected.

The discussions on these proposed modifications brought up a number of issues concerning the design of the studies, controls, questionnaires, possible selection bias, observation bias, sample size, etc, that are being considered by the investigators, PAHO and EPA officials.

5. Interviewers

During January and February while Dr. Nubia Munoz was visiting in Colombia, 15 interviewers, 12 of them with professional experience in national morbidity surveys, were invited to the National Institute of Health. Eight were selected and began training in early February. However, the performance of one of them was not considered satisfactory and presently the interviewing of the floriculture worker is being undertaken by seven professional interviewers.

This activity was scheduled to begin in February, but could be initiated only in mid March because of lack of transportation. The authorization for the purchase of the vehicle was transmitted by EPA to PAHO in December 1980 and although the purchase request was processed immediately, the manufacturers are unable to deliver it before the end of May 1981. The Colombian Principal Investigators were able to obtain a car on loan from the Ministry of Health, but it required extensive repairs which were completed only in March. Nevertheless, by 31 March 1981, the interviewers had covered approximately 50% of the companies. The results and more details concerning this activity will be provided upon completion and in the next semiannual report.

II. ADMINISTRATIVE PROGRESS REPORT

During 1-5 December 1980, Ms. Magaly Henson, Administrative Officer at PAHO, visited Bogota in order to discuss and establish with the PAHO Country Representative and the Principal Investigators of the National Institute of Health, the most suitable mechanisms for the administration and coordination of the study. Meetings were held with the Director of the National Institute of Health (INS) the Principal Investigators, Directors of Research and of Health of the Ministry of Health, the PAHO Representative in Colombia and other technical and administrative staff from the three institutions involved.

The initial project plan had been approved by the Minister of Health two years ago. Consequently, the local health authorities had no objections to the final version of the project as agreed upon by PAHO and EPA. Furthermore, in order to facilitate the development of the study the Ministry of Health, the Agency with direct responsibility over the National Institute of Health and for coordinating PAHO activities in the country, agreed to direct management relations between PAHO and the INS. In consequence PAHO and INS signed an agreement corresponding to the first year of the study. Also, in order to facilitate the payment of salaries of all personnel--long term and short term--acquisition of equipment and materials, maintenance of vehicles, etc., the PAHO office in Colombia agreed to provide administrative support for the disbursement and control of all funds including those under the sub-contract. The INS and PAHO in Bogota worked out a disbursement and accounting system that is functioning with very few delays.

Late in November 1980, Mr. John Hunter, Chief of Accounts in PAHO, taking advantage of a trip to Copenhagen, discussed with the Chief of Administration of IARC, Mr. K.G. Saita, a mutually acceptable system for the periodic reimbursement of IARC's expenses through WHO's general accounting system. The travel of Mr. Hunter was at no cost to the Project.

Also in late October, Dr. Allen Linsell, Director, Division of Epidemiology and Biostatistics of IARC, visited PAHO to discuss and define IARC's responsibilities in terms of the signed contract as well as the administrative procedures for travel of the Investigators, meetings, reporting of results. The costs of the visit of Dr. Linsell were also charged to sources other than the Contract.

In addition we have had the visit of Dr. Nubia Munoz on several occasions that her duties and responsibilities brought her to America. With the exception of Dr. Munoz visit to Colombia and Miami and February 1981, all of the other visits were made at no cost to the Contract.

Dr. John Davies of the University of Miami (U.M.) visited PAHO last August to discuss the project, the technical and administrative responsibilities of the U.M. and the budget. That visit has been followed by several telephone conversations. Expenses of the University of Miami have been limited to the travel of Drs. Davies and Levine to Bogota. Travel of Dr. Pfaffenberger was supported with other funds from the University of Miami. Although, the UM is programmed to receive financial support only beginning 1 June 1981, they have already given considerable effort and expenses to the project. The U.M. investigators have collected and analyzed preliminary blood and urine samples and are preparing the required standards for ETU analysis (S-butyl derivative) which required the efforts of co-investigators purchase of chemicals, supplies, literature references, etc.

In summary, the administrative and coordination aspects of the project are running satisfactorily. PAHO project officers are in frequent telephone communications with all the principal investigators and particularly the PAHO office in Colombia in order to ensure smooth running of planned activities and to help solve a few of the difficulties encountered in the purchase of the vehicle, materials, recruitment of interviewers, etc.

Finally, the close contact maintained by the EPA Project Officer, Ms. Jane Keller, with PAHO officers, and the cooperation of Dr. John Luecke, from NCI, who attended several of the periodic review meetings held, have been most helpful in the development of activities and in clarifying certain aspects of the Contract. Ms. Keller's interventions in the discussion in the Miami meeting and her interest in seeking outside opinions will, indeed, be very valuable to the success of the project.

III. FUTURE ACTIVITIES

During the coming months, through December 1981, the program of work includes in the teratogenesis study the completion of the survey to identify cases and controls, the study of the cases and control and of the planning for the prospective study.

One of the most important events in the near future is the appointment of a Scientific Review Committee. As preliminary discussed with Ms. Jane Keller from EPA, the Committee will serve as an advisory group for the purpose of suggesting modifications in the study design that could improve the final results. Membership will consist of independent experts on the different disciplines involved in the study--teratogenesis, carcinogenesis, epidemiological research, pesticide exposure, laboratory analysis--and who are familiar with the circumstances in Latin America. The Committee is to be convened by NCI in collaboration with PAHO.

ATTACHMENT I

LIST OF PESTICIDES USED IN THE FLORICULTURE INDUSTRY OF BOGOTA IN 1969

Rank	Names	Usage (Kg/Yr)
1	Captan	23,550
2	Mancozeb (Manzate 200)	10,760
3	Sodium Metham	8,670
4	Propineb (Mezineb, Antracol)	8,490
5	Metil isotiocianato+DD	5,290
6	Chlorothalonil (Daconil)	4,800
7	Indosulfan (Thiodan)	3,830
8	Tetradifon (Tedion)	3,570
9	Aldicarb (Temik)	2,840
10	Zineb	2,840
11	Quintozene (PCNB)	2,670
12	Benomyl (Benlate)	2,670
13	Picofol (Kelthane)	2,300
14	Malathion	2,230
15	Methyl oxydemeton (Metasystemox)	1,600
16	Carbaryl (Sevin)	1,460
17	Methomyl	1,440
18	Diazinon	1,430
19	Maneb	1,360
20	Ometeato (Folimat)	1,140
21	Oxicloro de cobre	1,090
22	Ethoprop (Mocap)	1,080
23	Phosphamidon (Dimecron)	1,050
24	Dibrom (Naled)	1,040 (?) 158 (?)
25	Disulfoton	980
26	Daromet	960
27	Difolatan (captafol)	960
28	Azufre	800
29	Aldrin	890
30	Cypermetrin	880
31	Carbofuran	800
32	Alquil-aril polieter alcohol	760
33	Oxicarboxin	710
34	Pirimicarb	710
35	Dimetoato	700
36	Dicrotofos	670
37	Triforina	660

Rank	Names	Usage (Kg/Yr)
38	Bis (pentacloro 2-4 ciclopentadien)	580
39	Parathion	540
40	Forato	530
41	Lindano	500
42	Paraquat	460
43	Ferban	430
44	Metilparathion	420
45	Metamidotos	420
46	Tiometon	370
47	Acido fNaffalen aretico	340
48	Acefato	330
49	Cobre metalico	330
50	Mevinfos	330
51	Hidroxido Triciclohexil estano	310
52	Monocrotofos	320
53	Dodemorf acetato	300
54	Dicloran (ditramil)	290
55	Fenovalerato	260
56	Carbendacim	250
57	Formotion	250
58	Decametrin	240
59	Tiabendazol	220
60	Binopacrilo	210
61	Acetato de vinilo	200
62	Triclorfon	200
63	Metaldehido	190
64	Diclofluanid	180
65	Naled	160
66	Canfecloro	150
67	Demeton	150
68	Heptacloro	150
69	Permethrin	120
70	Fenamifos	110
71	Kasugamicin	90
72	Triclorofluorometano	90
73	Dinocap	60
74	Clororo de mercurio	50
75	Piperalin	50
76	Triclorofenol	50
77	Ditriciclohexil hidroxido de estano	40

Rank	Names	Usage (Kg/Yr)
78	Glifosato	40
79	Isopropil 2-cloroetil sulfito	40
80	Profenofos	40
81	Triozofos	40
82	Bis (p.-clorofenil)-3-piridiometanol	40
83	Dalapan	30
84	Oxamyl	30
85	Triodimefon	30
86	Clorodifluorometano	20
87	Oxithioquinox	20
88	Tridemorph (Calixin)	20
89	Diclorvos	12
90	Morfation	10
91	Diuron	10
92	Atrozina	9
93	Etefon	5
94	Bromuro de metil	2
95	Pirazofos	1

ATTACHMENT II

FLORICULTURE PESTICIDES USED IN EXCESS OF 1,000 kg/Year (1969)

Rank	Names	Usage (Kg/Yr)
1	Captan	23,550
2	Mancozeb (Manzate 200)	10,760
3	Sodium Metham	8,670
4	Propineb (Mezineb, Antracol)	8,490
5	Chlorothalonil (Daconil)	4,800
6	Endosulfan (Thiodan)	3,830
7	Tetradifon (Tedion)	2,840
8	Aldicarb (Temik)	2,840
9	Zineb	2,840
10	Quintozene (PCNB)	2,670
11	Benomyl (Benlate)	2,670
12	Dicofol (Kelthane)	2,300
13	Malathion	2,230
14	Methyl oxydemeton (Metasystemox)	1,600
15	Carbaryl (Sevin)	1,460
16	Methomyl	1,440
17	Diazinon	1,430
18	Maneb	1,360
19	Ometeato (Folimat)	1,140
20	Ethoprop (Mecap)	1,080
21	Phosphamidon (Dimecron)	1,050
22	Dibrom (Naled)	1,040

ATTACHMENT III

Table 1: Frequency distribution by age and sex of workers in Bogata's floriculture

Age in Years Sex	15	16-20	21-25	26-30	31-35	36-40	41-45	45	Without info.	TOTAL
Male	7	667	862	526	372	220	159	287	100	3.200
Female	4	1.370	1.673	1.021	578	437	207	100	369	5.759
TOTAL	11	2.037	2.535	1.547	950	657	366	387	469	8.959

Table 2: Frequency distribution of workers according to duration of work in Floriculture

Time (Months)	12	12-24	25-36	37-48	49-60	61-72	72	Without info.	TOTAL
Number of workers	2,533	2.400	1,207	709	430	274	1.128	278	8.959
%	28.2	27.7	13.4	7.9	4.7	3.0	12.5	3.1	100
Accumulated %	28.2	55.9	69.3	77.2	81.9	84.9	97.4	100	

Table 3

DISTRIBUTION BY AGE AND SEX OF PESTICIDE FORMULATORS IN COLOMBIA

Age in years Sex	15-20	21-25	26-30	31-35	36-40	41-45	45	Without info.	Total
Male	8	53	112	78	59	28	36	63	437
Female	3	1	0	1	0	1	0	14	20
TOTAL	11	54	112	79	59	29	36	77	457

TABLE 4

DISTRIBUTION OF PESTICIDE FORMULATORS IN COLOMBIA BY LENGTH OF STAY AT WORKPLACE

Time (months)	12	12-24	25-36	37-48	49-60	61-72	72	Without info.	Total
Number of workers	45	44	87			28	195	1	457
					.6	6.1	42.6	0.2	100
Accumu-lated	9.		.	.	.	56.8	99.4	99.6	100

TABLE 5

DISTRIBUTION BY AGE OF THE SANITARY CAMPAIGN WORKERS IN COLOMBIA

Age (Years)	15-20	21-25	26-30	31-35	36-40	41-45	45	Without info.	Total
Workers	4	71	136	110	59	40	21	264	701

TABLE 6

DISTRIBUTION BY LENGTH OF STAY AT WORKPLACE OF SANITARY CAMPAIGN WORKERS IN COLOMBIA

Time (months)	12	12-24	25-36	37-48	49-60	61-72	72	Total
Number of workers	63	213	153	52	50	49	92	701
%	8.9	30.3	21.8	7.4	7.1	6.9	13.1	100
Accumulated	8.9	39.2	61	68.4	75.5	82.4	95.5	100

ATTACHMENT II

Colombian Urines - Alkyl Phosphate Data

No.	DMTP (ppm)	DETP (ppm)	DMP (ppm)	DEP (ppm)
1	ND	ND	ND	ND
2	0.02	ND	ND	ND
3	ND	ND	ND	ND
4	ND	ND	ND	ND
5	0.02	0.02	ND	0.02
6	0.02	0.11	1.13	0.15
7	ND	0.04	ND	0.04
8	ND	ND	2.47	ND
9	0.02	0.02	ND	ND
10	ND	ND	ND	ND

[a] probably multiple exposure to OP's.

[b] probably exposure to either Dimecron or Naled.

Colombian Serums - OC and OP Data

No.	pp'-DDE (ppb)	pp'DDT (ppb)	Dieldrin (ppb)	HCB (ppb)	b-BHC (ppb)	OP
1	38.2	4.2	1.4	1	1.1	ND
2	24.0	2.4	1.8	1	ND	ND
3	20.2	2	1	Int	ND	ND
4	16.5	2	2.5	1	1	ND
5	12.2	2.3	1.3	1	1	ND
6	37.9	2	1	1	1	ND
7	13.1	3.3	2.6	1	ND	ND
8	37.9	5.5	1.0	1	1	ND
9	24.6	2.9	1.2	1	1	ND
10	(Broken)	----	----	----	----	----
Mean:	24.9	2.5	----	----	----	----

SPEAKER: You mentioned 388 malformed children. Was that in the case control cohort? Because you had an expected number, in the abstract at least, of 134. That would make a tremendous difference. Is that a correct assumption?

DR. TORLONI: Yes, you picked up the right figure. That is what has been reported on the 8,650 questionnaires that have been supplied to workers. When they are in the process of punching this material, they have done a very preliminary analysis, right there in Colombia. And they identified among these 8,650 that number of malformations and 14 tumors.

Now, we have been informed in another report, that 100 of these children have already been subjected to a very thorough medical examination by a pediatrician who has been trained. And they are also doing some genetic studies. We don't have the data of the results of these 100 medical checks of these children.

Also, as a pathologist, we have suggested to them to put some more emphasis on a better qualification of what kind of malformation are we talking about. If these children have died and you have access to autopsy, or if you have surgical biopsy, or whatever, there should be a parallel study that will help to confirm and prove scientifically some of the clinical impressions. Unfortunately, at this stage I don't have access to this information. But the number is 388.

PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Biomedical and Histological Correlates of Chloroform Carcinogenesis in the Mouse and Rat--Status Report

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INTRODUCTION

There have been several studies of tumorigenesis induced in animals by chloroform. Eschenbrenner (1945) reported hepatomas induced in female Strain A mice receiving 0.3 or 0.6 mg/kg of chloroform by gavage every four days for three months. In a study conducted by Hazleton Laboratories (1976) for the National Cancer Institute, chloroform was administered by gavage five times per week for 78 weeks to Osborne-Mendel rats at 90-250 mg/kg/day and to B6C3F1 mice at 100-500 mg/kg/day. Kidney epithelial tumors were induced in male rats and hepatocellular carcinomas in mice of both sexes. Decreased survival rates and body weights were reported in treated rats but were not observed in the mice. Theiss et al. (1977) reported no increase in lung adenomas in Strain A mice recieving intraperitoneal injections of chloroform at 80 or 200 mg/kg three times per week for 8 weeks, or two injections at 400 mg/kg.

The present study was designed to evaluate the chronic toxicity and tumorigenicity of chloroform administered in the drinking water to male Osborne-Mendel rats and to female B6C3F1 mice over a wide range of dose levels.

METHODS

Animals

The species and strains of rodents used were those in the earlier study conducted by Hazelton. Rats were received from CAMM Research, and mice from Charles River. Mice were housed five per cage in 48.3 x 26.7 x 15.2 cm (19" x 10½" x 6") polycarbonate cages containing hardwood chip bedding (AbSorbDri). Cages were changed once a week at the same time as the body weights and water consumption were recorded.

Rats were housed two per cage in 47.6 x 26.0 x 20.3 cm (18-3/4" x 10½" x 8") polycarbonate cages containing the same hardwood chip bedding. Cages were changed twice a week--once when body weights and water consumption were recorded and again when water consumption was recorded for the second time in the week.

Animals were assigned to experimental groups and cages using a table of random numbers.

For both species, cages were rotated on the racks once a week. The racks were rotated on the same schedule within the room to ensure similar conditions for all animals throughout the study.

Control animals were housed in rooms as similar as possible but separate from the chloroform-treated groups.

Chemical Supply

The chloroform used in this program was pesticide quality purchased from Matheson Coleman Bell. The chloroform was analyzed for purity, concentration, and stability in the drinking water. Before use, all chloroform was distilled to remove contaminants (such as diethyl carbonate and ethanol). Glass distilled water was used throughout the study for control drinking water as well as for the chloroform solutions.

Analytical Chemistry

Quantitative analyses for chloroform in air, water, food and blood; distillation of chloroform and determination of purity levels; determination of liver fat/organ weight ratios; and routine monitoring of feed lots for PCBs and chlorinated pesticides were performed.

Ambient air in each experimental room was analyzed once every six months for chloroform concentrations. The Purina Laboratory Chow was analyzed annually for chloroform concentrations. Additionally, samples from water bottles were analyzed once each month on the Friday following the stock solution analysis. Liver fat/liver weight ratios were examined at 3 and 6 months in both rats and mice.

Dose Levels-Numbers of Animals

The negative control (0 ppm) and 200 ppm rat groups were each comprised of 330 animals. There were 150 rats in the 400 ppm groups and 50 in the 900 ppm, 1800 ppm, and matched control (M) groups. The negative control and 200 ppm mouse groups each had 430 animals, while the numbers of mice in the other groups were identical to the corresponding rat groups.

In addition to the above numbers of animals, there were 80 additional male rats per group for blood work and 10 additional male rats in all but the matched control group for liver fat analyses at 90 days on test. With the exception of the matched control group, each of the mouse groups had 20 additional mice. Ten per group were removed at 3 months and ten per group were removed at 6 months for liver fat analyses.

Observations

Food intake and animal health and activity were noted daily, 7 days a week. Body weights were recorded weekly until stable (18 wekes for the rats and 19 weeks for the mice). Thereafter, body weight measurements were recorded monthly. Water consumption was measured twice weekly by weighing the water bottles during the two changes in water each week.

Hematology and Clinical Chemistry

The following blood parameters were evaluated on 20 male rats from each experimental group at 6, 12, 18, and 24 months on test.

Hematology	Clinical Chemistry	
Hematocrit	Triglycerides	Glucose
Hemoglobin	Total Bilirubin	BUN
Red cell count	SGOT	Creatinine
White cell count	SGPT	Uric acid
Differential count	LDH	Na^+
	Alkaline phosphatase	K^+
MCV	Total iron	CO_2
MCH	Total protein	Cl^-
MCHC	Albumin	Calcium
	Globulin	Phosphorus
	A/G Ratio	Balance (na-($Cl+CO_2$)
		Cholesterol

A Coulter Counter® Model S was used to determine red and white cell counts, hemoglobin, hematocrit, MCV, MCH, and MCHC. Differential counts were conducted manually by a trained technologist. The chemical analyses were run on a Sequential Multiple Analysis Plus Computer (SMAC) system.

These animals were handled in the same manner as those in the lifetime study. At time of sacrifice for blood analyses, we performed a gross necropsy and collected and saved the appropriate tissues in 10% neutral buffered formalin. No histopathology will be conducted on these animals unless the terminal sacrifice data indicate a need to review specific tissues at stages on test earlier than those obtained from planned sacrifices or from animals that die during the study. A review of liver and kidney lesions and their subsequent correlation with hematology and clinical chemistry findings will be conducted on an individual animal basis then correlated by treatment group.

Gross and Microscopic Pathology

When any animal in these lifetime studies appeared unlikely to survive until the next scheduled observation, it was sacrificed. This aggressive sacrifice was intended to prevent the loss of information that could occur through autolysis or cannibalization.

All decedents were given a complete gross necropsy as defined in the Guidelines for Carcinogen Bioassay in Small Rodents: external examinations, including body orifices, and examination and fixation of all of the following:

Gross lesions
Tissue masses of suspect tumors and regional lymph nodes
Skin
Mandibular lymph node
Mammary gland
Salivary gland
Larynx
Trachea
Cecum
Colon
Rectum
Mesenteric lymph node
Liver
Thigh muscle
Sciatic nerve
Sternebrae, vertebrae, or femur (plus marrow)
Costochondral junction, ribs
Thymus
Gall bladder
Pancreas
Lungs and bronchi
Heart
Thyroids
Parathyroids
Estophagus
Stomach
Duodenum
Jejunum
Ileum
Spleen
Kidneys
Adrenals
Bladder
Seminal vesicles
Prostate
Testes
Ovaries
Uterus
Nasal cavity
Brain
Pituitary
Eyes
Spinal cord

All tissues and organs were fixed in 10% neutral buffered formalin.

The following tissues were processed and read histologically with all others being stored in fixative.

Suspect tumors and gross lesions
Liver
Kidney
Regional lymph nodes
Urinary bladder
Esophagus
Adrenals
Spleen
Stomach
Small intestine
Colon
Lung

All rat slides were read by one pathologist, and all mouse slides were ready by a second pathologist.

RESULTS

Since the histopathological examination of the tissues from this study is still in progress, the results presented here are necessarily incomplete. Nonetheless, they provide a basis for evaluating the progress of the study and some information about the chronic effects of chloroform.

Table 1 shows the results of the analyses of the chloroform solutions in the drinking bottles. Chloroform concentrations varied somewhat over the course of the study, but were generally within less than ten percent of the nominal values. Clearly, there were no overlapping values between adjacent dose groups.

Survival, water consumption, and body weight patterns for the rats are shown in Figure 1. No early deaths occurred and survival was essentially directly proportional to the chloroform dose level, lowest for the negative controls, next lowest for the 200 and 400 ppm groups, and highest for the matched controls and the 900 and 1800 ppm groups. Water consumption increased slowly over the course of the study in all groups, with the amount consumed inversely proportional to dose level during the first 18 months, then a clustering of the negative control, 200 adn 400 ppm groups at a higher level, the 900 ppm group in the middle, and the lowest consumption shown by the 1800 ppm group. Body weight patterns have shown typical growth curves, the values at any time point being inversely proportional to dose level. Values for the matched control group were above those for the 1800 ppm group during the first year, but the latter group has now caught up with their matched controls.

Survival, water consumption and body weight patterns for the mice are illustrated in Figure 2. Unlike the rats, there was significant early mortality in the treated mice, particularly at 900 and 1800 ppm. From the second through the eighteenth month very few animals died, but by the twenty-third month some mortality, proportional to odse level, was apparent. Both water consumption and body weights were narrowly distributed among the various groups, and the values for the two extreme groups, the negative controls and the 1800 ppm group, are shown for illustration. Water consumption for the mice was essentially stable throughout the study. Body weight increased more or less linearly, then reached a plateau beginning at about eighteen months.

Although mean blood levels of chloroform were assessed in the rats at 3, 6, 12, and 18 months, there was difficulty in obtaining reliable data during the first year. For the 18- and 24-month assays, whole blood was used instead of serum, and reliable results were obtained. At 18 months the values were 0.7, 0.8, 7.5, 22.1, 75.4, and 124 ppb for the negative controls, matched controls, and the 200, 400, 900, and 1800 ppm groups, respectively. The 24-month values were 0.4, 0.2, 18.2, 13.8, 80.8, adn 135 ppb for the negative controls, matched controls, and the 200, 400, 900, and 1800 ppm group, respectively.

The percent fat in the liver of the rats and the mice at 3 and at 6 months is shown in Table 2. For the rats, there was no apparent increase in liver fat content in the treated groups at 3 months, but at 6 months there was a significant increase in the 1800 ppm group. In themice, significant increases in liver fat were apparent at 400-1800 ppm at 3 months and in all treated groups at 6 months.

The hematologic findings in the rats at 6, 12, and 18 months are shown in Tables 3-6. WBC values were lower in the 1800 ppm group and in the matched controls at 6 and at 12 months. The differences in erythrocyte and hemoglobin parameters at 12 months are consistent with hemoconcentration in the treated groups, but no significant differences were apparent at 18 months.

The blood chemistry data for male rats sacrificed at 6, 12, and 18 months are shown in Tables 7-10. Although various parameters differed significantly from the negative control values at some time points, there were a number of apparent trends. Potassium, phosphorus, bilirubin, alkaline phosphatase, total iron, albumin, and the albumin/globulin ratio tended to be higher in treated groups than in the negative controls. Chloride, cholesterol, triglycerides, LDH and globulin tended to be lower in treated groups than in the negative controls. In the matched controls, glucose and total iron tended to be higher and cholesterol and triglycerides tended to be lower than in the negative controls.

Histopathologic evaluations have been completed for 200 rats and 62 mice that were sacrificed or found dead during the first 20 months of the study. From the mortality data, it is apparent that these samples are highly biased both in time and toward negative control animals. The percentages of rats examined to date are 45, 12, 10, 5, 6, and 4% for the negative control, matched control, 200, 400, 900, and 1800 ppm groups, respectively. Corresponding percentages for the mice are 3, 4, 4, 6, 20, and 18% (including early deaths). Thus, assessment of the effect of chloroform on tumorigenesis is not possible at this time.

For the rats, the following conditions in the indicated sites have been observed in appreciable numbers:

Adrenal cortex--adenoma, hyperplasia, hypertrophy, vacuolation

Adrenal medulla--pheochromocytoma, hyperplasia

Kidney--cyst, calculi, glomerulonephritis, hydronephrosis

Lung--arterial, hypertrophy, atelectasis, congestion, interstitial pneumonia

Lymph nodes--hemorrhage

Parathyroids--hyperplasia

Spleen--hematopoiesis, hemosiderosis

Stomach--mineralization

In addition, sporadic tumor diagnoses have been made in the rats in various organs, including disseminated lymphsarcoma, and adenocarcinoma in the GI tract; squamous cell carcinoma, undifferentiated sarcoma and sebaceous gland adenoma in the ear; fibrosarcoma in the heart; tubular carcinoma, clear cell adenoma, adenocarcinoma, transitional cell carcinoma and hemangiosarcoma in the kidney; bronchiolar adenoma in the lung; osteosarcoma, fibroma sarcoma, fibrosarcoma, fibroadenoma and hemangiosarcoma in various body regions; squamous cell carcinoma in the nostril; carcinoma in the nasal cavity; parathyroid adenoma; basophilic carcinoma in the pituitary; adenocarcinoma in the prostate; squamous cell carcinoma and papilloma in the stomach; interstitial cell tumor in the testes; and carcinoma, cystadenoma and adenoma in the thyroid.

There have been insufficient mice examined to identify any lesions of appreciable incidence. Sporadic tumors in the mice have been diagnosed as disseminated lymphoma, both histiocytic and lymphocytic; pheochromocytoma; alveolar/bronchiolar adenoma and carcinoma; mammary adenoma and adenocarcinoma; carcinoma and teratoma in the ovary and leiomyocarcoma in the uterine cervix.

DISCUSSION

With administration of chloroform at constant levels in the drinking water, the daily doses received by the animals are a function of fluid consumption and body weight. Using the water consumption and body weights for the first and twenty-third months for illustration, the calculated mean daily doses for the rats receiving 200, 400, 900, or 1800 ppm of chloroform were 34, 66, 143, and 305 mg/kg and 34, 69, 132, and 238 mg/kg, during Months 1 and 23, respectively. For the mice, the corresponding values were 54, 95, 207, and 382 mg/kg and 31, 63, 150, and 309 mg/kg. Thus, the daily doses in the present study were comparable to those used in two previous studies (Hazleton Laboratories, 1976; Theiss, 1977) at the higher levels and extended below them at the lower levels.

Assuming that the water consumption and body weight data provide indices of food consumption, the increased survival and decreased body weight in rats receiving chloroform are consistent with the well-known increase in longevity associated with caloric restriction (McCay, 1947). Since caloric restriction also results in reduced age-specific incidences of most pathologic conditions associated with aging (McCay, 1947), it will be interesting to consider the pathologic findings from the present study when they become available. It is also evident that inclusion of matched controls in long-term studies is extremely important.

In the mice, the effect of chloroform on survival was markedly different than in the rats. It appears that some of the mice rejected the chloroform solutions during the first weeks to such a degree that they were unable to survive. In fact, the only behavioral effect of the chloroform that was discernible during this study was the unthrifty appearance of the mice during this time. From the water consumption and body weight data, it is clear that caloric restriction in the mice surviving the first weeks occurred to a much lesser extent than in the rats, if at all.

The increase in liver fat content for the mice was clearly greater than for the rats. It is probable that, in addition to differences between the mouse and the rat in the metabolism of chloroform (Butler, 1961; Paul et al., 1963), the greater caloric restriction in the rats may have been a factor in reducing the relative accumulation of fat in the liver.

While the trends observed in some of the blood chemistry parameters associated with chloroform administration in the rat may be related to a nephrotoxic effect of the chloroform, nephropathy is one of the findings in the negative controls, and no definitive statements about the nephrotoxic effects of chloroform in the present study can be made at this time.

Discussion of the gross necropsy and histologic information will have to be delayed until the terminal sacrifices and the histopathologic evaluations have been completed.

HEMATOLOGY AND CLINICAL CHEMISTRY

Hematology

- White cell count
- Red cell count
- Hemoglobin
- Hematocrit
- MCV
- MCH
- MCHC

Differential count

- PMN
- Bands
- Lymphocytes
- Monocytes
- Eosinophils
- Basophils

Clinical Chemistry

- Glucose
- BUN
- Creatinine
- Uric acid
- Na^+
- K^+
- CO_2
- Cl^-
- Calcium
- Phosphorus
- Balance, $Na-(Cl+CO_2)$
- Cholesterol
- Triglycerides
- Total Bilirubin
- SGOT
- SGPT
- LDH
- Alkaline phosphatase
- Total iron
- Total protein
- Albumin
- Globulin
- A/G

TABLE 1

Measured Chloroform Concentrations in Water Bottles In Percent of Nominal Values

(Mean of ten samples per level per month)

	Chloroform Level (ppm)			
Month	200	400	900	1800
1	93	94	88	76
2	84	93	95	94
3	104	108	80	94
4	92	84	89	89
5	99	102	100	99
6	106	94	90	73
7	106	100	101	98
8	107	97	96	98
9	103	104	105	103
10	104	104	100	94
11	95	96	91	88
12	89	88	88	83
13	96	98	94	97
14	90	95	92	89
15	100	104	100	97
16	108	100	100	105
17	100	103	121	96
18	91	87	95	94
19	104	102	101	98
20	104	104	101	105
21	104	100	107	96
22	92	93	90	79
23	105	100	110	109
24	117	111	109	99

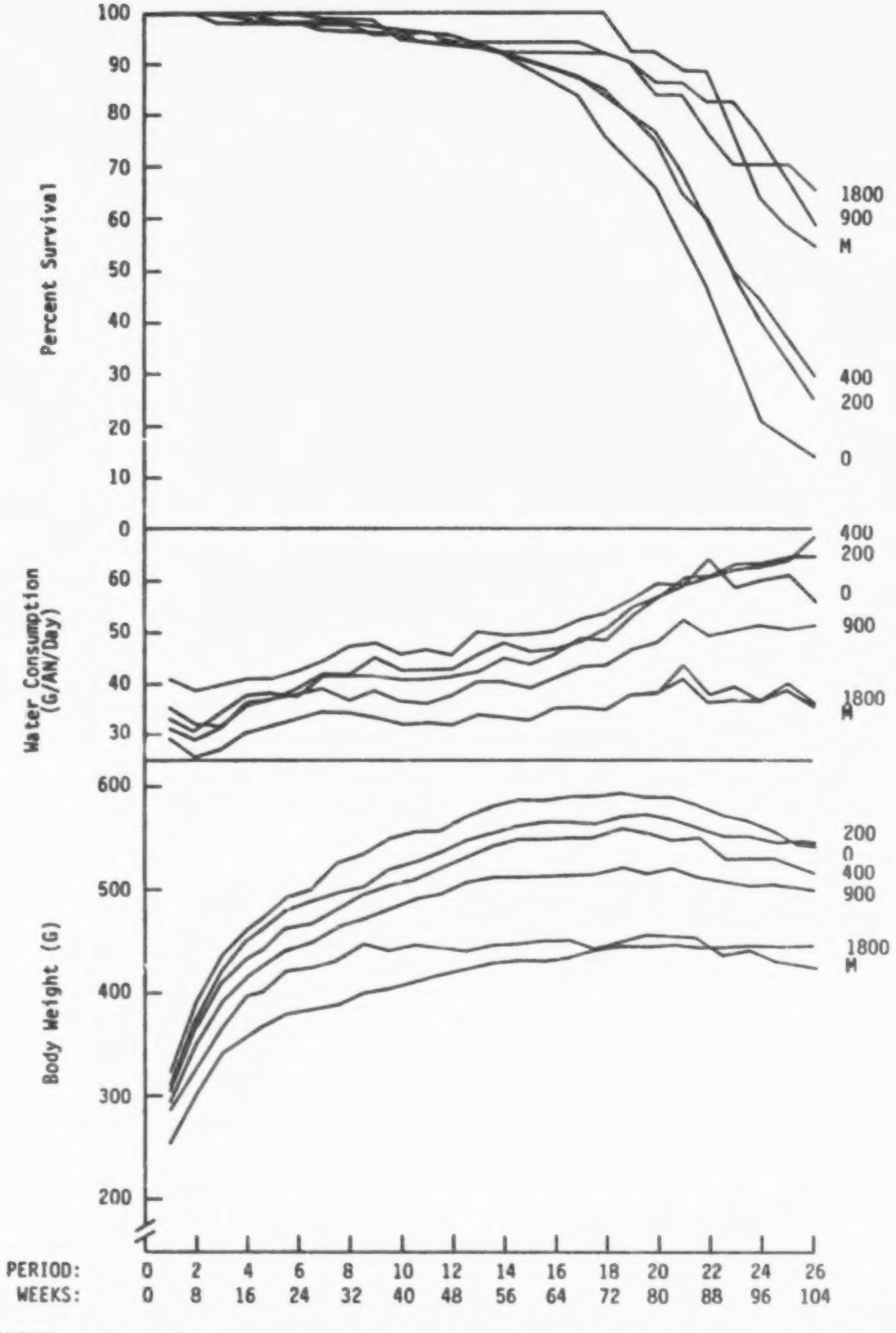

FIGURE 1 Survival, water consumption and body weight in male Osborne-Mendel rats receiving chloroform in the drinking water. Doses are indicated in ppm, M indicates matched control group receiving an amount of water equal to that consumed by the 1800 ppm group.

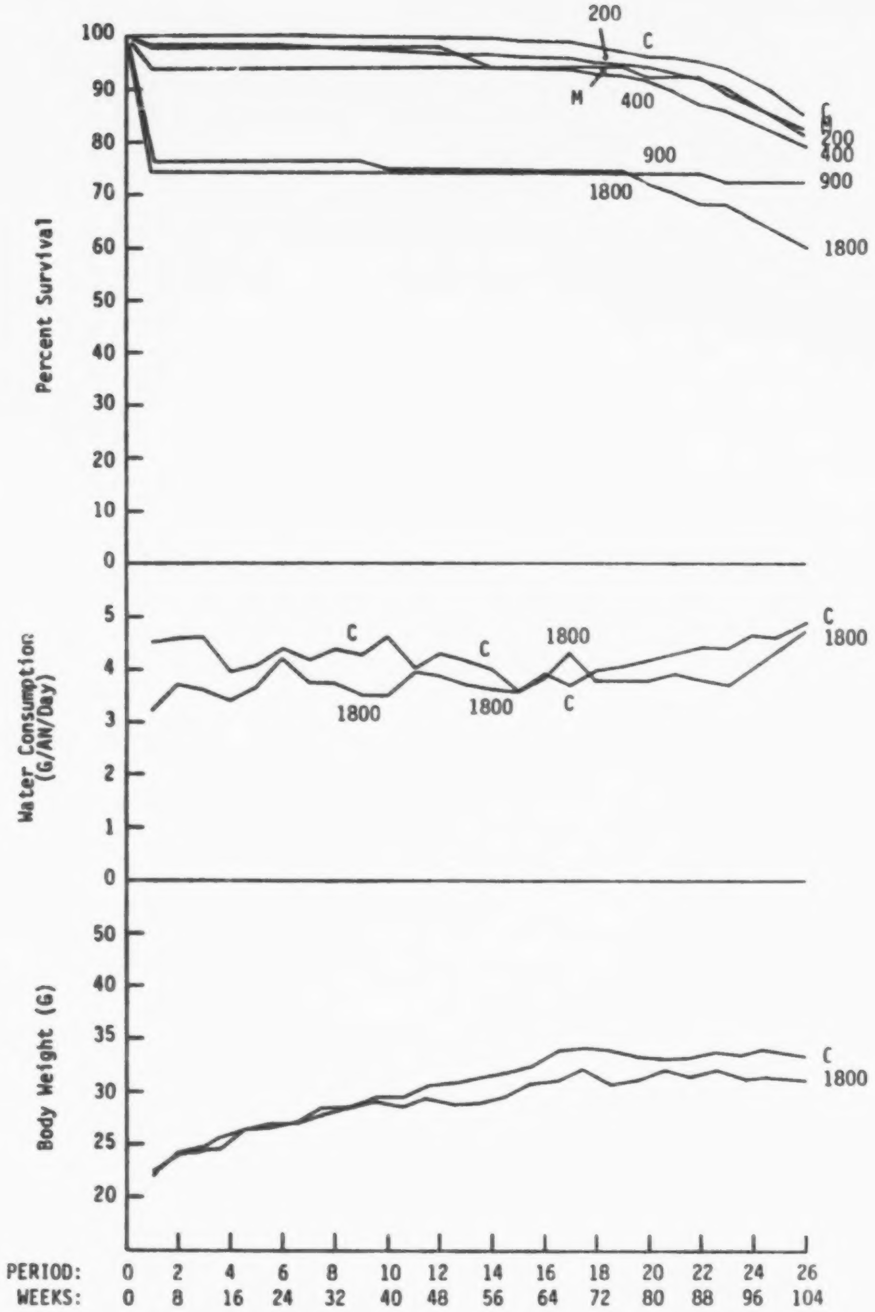

FIGURE 2 Survival, water consumption and body weight in female B6C3F1 mice receiving chloroform in the drinking water. Doses are indicated in ppm, M indicates matched control group receiving an amount of water equal to that consumed by the 1800 ppm group.

TABLE 2

Mean Percent Liver Fat In Animals Receiving Chloroform In the Drinking Water

	Dose Group	Time on Study			
		3 Months		6 Months	
		N	%	N	%
Rats	0 ppm	10	3.3	20	4.5
	M	--	--	18	4.6
	200 ppm	10	3.3	20	4.5
	400 ppm	10	3.2	20	4.6
	900 ppm	10	3.6	19	4.8
	1800 ppm	10	3.5	19	5.1[1]
Mice	0 ppm	10	3.3	7	5.8
	200 ppm	10	3.5	10	7.9[1]
	400 ppm	10	3.9[2]	10	6.8
	900 ppm	10	4.5[2]	6	7.1[1]
	1800 ppm	8	6.4[2]	8	10.4[2]

[1] $p \le 0.05$.

[2] $p \le 0.01$.

TABLE 3

Summary of Hematology Data For Male Osborne-Mendel Rats Receiving Chloroform In Their Drinking Water

6-Month Sacrifice

Parameter	Control	Matched Control	Chloroform (ppm) 200	400	900	1800
WBC x $10^3/mm^3$	6.8	4.8[2]	6.6	6.5	6.8	5.5[1]
RBC x $10^6/mm^3$	8.06	8.14	8.19	8.07	8.04	7.98
Hgb, gm%	15.3	15.4	15.5	15.3	15.2	15.3
Hct, %	44.2	44.3	44.4	43.6	43.7	44.0
MCV, μ^3	55	55	54	54	55	55
MCH, $\mu\mu g$	18.9	18.7	18.8	18.7	18.8	19.0
MCHC, %	34.7	34.7	35.0	35.0	34.9	34.8
Differential (%)						
PMN	10.7	14	13	12	11	12
Bands	0	0.1	0.1	0	0.1	0
Lymphocytes	85	82	84	85	86	84
Monocytes	2.5	2	2	2	2	2
Eosinophils	2	2	1.6	1	1	2
Basophils	0	0	0	0	0	0

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[1] $p \leq 0.05$.

[2] $p \leq 0.01$.

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TABLE 4

Summary of Hematology Data for Male Osborne-Mendel Rats Receiving Chloroform In Their Drinking Water

One-Year Sacrifice

Parameter	Control	Matched Control[1]	Chloroform (ppm)			
			200	400	900	1800
WBC x $10^3/mm^3$	6.7	5.9[3]	6.6	6.4	5.0[3]	4.4[3]
RBC x $10^6/mm^3$	7.84	7.98	8.17[3]	7.97	8.31[3]	7.97
Hgb, gm%	14.9	15.2	15.3[2]	15.2	15.6[3]	15.4[2]
Hct, %	43.1	44.6[2]	43.4	44.4	45.1[3]	44.4[2]
MCV, μ^3	55	56	53[3]	56	55	56
MCH, μμg	18.9	18.9	18.7	19.0	18.7	19.2
MCHC, %	34.4	33.9	35.3[2]	34.0	34.5	34.5
Differential (%)						
PMN	20	19	16	14	17	16
Bands	0	0	0	0	0	0
Lymphocytes	78	79	81	84	80	81
Monocytes	2	1	1	1	2	1
Eosinophils	1	1	1	1	2	2
Basophils	0	0	0	0	0	0

[1] One sample clotted, therefore, only 19 samples were analyzed for this level. All other levels contained 20 samples. The differential count for this level contained 20 samples.

[2] $p \le 0.05$.

[3] $p \le 0.01$.

TABLE 5

Summary of Hematology Data for Male Osborne-Mendel Rats Receiving Chloroform In Their Drinking Water

18-Month Sacrifice

Parameter	Control	Matched Control[1]	Chloroform (ppm)			
			200	400	900	1800
WBC x $10^3/mm^3$	6.6	6.2	6.0	6.7	5.8	6.4
RBC x $10^6/mm^3$	7.43	7.57	7.38	7.66	7.76	7.49
Hgb, gm%	14.4	14.7	14.4	15.0	14.9	14.4
Hct, %	41.6	42.8	41.4	42.8	42.5	41.5
MCV, μ^3	56	57	57	56	55	56
MCH, $\mu\mu g$	19.4	19.4	19.5	19.6	19.2	18.7
MCHC, %	34.8	34.3	34.7	35.2	35.0	34.7
Differential (%)						
PMN	21	21	19	21	20	27
Bands	0	0	0	0	0	0
Lymphocytes	74	76	77	75	77	69
Monocytes	3	2	2	3	2	2
Eosinophils	2	2	2	1	1	2
Basophils	0	0	0	0	0	0

[1] One sample clotted, therefore, only 19 samples were analyzed for this level. All other levels contained 20 samples. The differential count for this level contained 20 samples.

Table 6

SUMMARY OF HEMATOLOGY DATA FOR MALE OSBORNE-MENDEL RATS RECEIVING CHLOROFORM IN THEIR DRINKING WATER

Two-Year Sacrifice

Parameter	Control	Matched Control	Chloroform (ppm) 200	400	900	1800
WBC x $10^3/mm^3$	11.0	5.5	6.1	5.3	5.4	6.0
RBC x $10^6/mm^3$	7.08	7.34	7.17	7.47	7.92	7.57
Hgb, gm%	14.0	14.8	14.3	14.9	15.5	15.2
Hct, %	39.1	40.8	39.3	40.9	42.8	42.2
MCV, μ^3	55	55	55	55	54	56
MCH, μμ	19.7	20.0	20.0	19.8	19.5	19.9
MCHC, %	35.7	36.1	36.3	36.3	36.2	35.8
Differential (%)						
PMN	33	26	31	32	24	29
Bands	0	0	0	0	0	0
Lymphocytes	64	72	66	63	71	68
Monocytes	2	1	2	2	2	2
Eosinophils	1	2	1	3	3	2
Basophils	0	0	0	0	0	0

TABLE 7

Summary of Clinical Chemistry Data For Male Osborne-Mendel Rats
Receiving Chloroform In Their Drinking Water

6-Month Sacrifice

Parameter	Control	Matched Control	Chloroform (ppm) 200	400	900	1800
Glucose, mg%	182	215[1]	180	187	201	193
BUN, mg%	22	24.4[2]	21.6	20.6	21.2	26.3[2]
Creatinine, mg%	0.6	0.6	0.6	0.6	0.7	0.7[1]
Uric Acid, mg%	2.0	2.9	2.1	2.1	2.5	2.9
Na$^+$, meq/l	146	147	146	146	146	145
K$^+$, meq/l	5.3	5.9	5.2	5.4	5.6	6.4[2]
CO$_2$, meq/l	24.0	24.8	26.6	27.0	26.0	26.0
Cl$^-$, meq/l	99.7	96.3	99.7	100	102[2]	102[2]
Calcium, mg%	10.0	10.0	9.9	10.1	10.3[2]	10.5[2]
Phosphorus, mg%	5.9	5.8	6.0	6.1	6.7[2]	7.4[2]
Balance, Na-(Cl + CO$_2$)	22	20	20	18.5[2]	18[2]	18[2]
Cholesterol, mg%	111	89[1]	107	114	103	100
Triglycerides, mg%	127	69[2]	120	116	68[2]	32[2]
Total Bilirubin, mg%	0.13	0.13	0.13	0.13	0.17[1]	0.19[2]
SGOT, mU/ml	180	170	135	127	114[1]	129
SGPT, mU/ml	114	101	81	82	47	91
LDH, mU/ml	1828	1169[2]	1195[2]	994[2]	821[2]	744[2]
Alkaline Phosphatase, mU/ml	194	221	180[1]	173	165	197
Total Iron, μg%	182	193	170	188	209[2]	202
Total Protein, gm%	5.7	5.9	5.6	5.6	5.7	5.7
Albumin, gm%	2.8	3.0[1]	2.8	2.8	3.0[2]	3.1[2]
Globulin, gm%	2.8	2.9	2.8	2.8	2.7[1]	2.6[2]
A/G	1.0	1.05	1.0	1.05	1.1[2]	1.2[2]

[1] $p \leq 0.05$.

[2] $p \leq 0.01$.

TABLE 8

Summary of Clinical Chemistry Data For Male Osborne-Mendel Rats Receiving Chloroform In Their Drinking Water

One-Year Sacrifice

Parameter	Control	Matched Control	Chloroform (ppm)			
			200	400	900	1800
Glucose, mg%	182	240[1]	183	220	220	211
BUN, mg%	22	23	20[2]	21	21	24[2]
Creatinine, mg%	0.7	0.8	0.7	0.8	0.7	0.8
Uric Acid, mg%	2.3	3.1	2.0	3.5	3.5	3.1
Na^+, meq/l	146	149[2]	146	147	146	146
K^+, meq/l	5.1	6.3[1]	5.1	6.6[2]	6.6	7.0[1]
CO_2, meq/l	27	25	27	25	25	24[1]
Cl^-, meq/l	99	103[2]	101[2]	99	101[2]	103[2]
Calcium, mg%	10.6	10.5	10.4	10.8	10.9	10.7
Phosphorus, mg%	5.4	6.1[1]	5.2	6.3[2]	6.1[1]	6.7[2]
Balance, Na-(Cl + CO_2)	20	21	18[1]	22	21	19
Cholesterol, mg%	159	112[2]	146	169	127[2]	105[2]
Triglycerides, mg%	323	138[2]	257	249	91[2]	34[2]
Total Bilirubin, mg%	0.1	0.1	0.1	0.1	0.2[2]	0.2[2]
SGOT, mU/ml	204	221	136[1]	211	166	163
SGPT, mU/ml	112	117	100	156	145	105
LDH, mU/ml	1386	1380	1065[2]	1169	1059[1]	668[2]
Alkaline Phosphatase, mU/ml	167	148[1]	161	140[2]	174	218[2]
Total Iron, μg%	164	193[2]	181[1]	183[1]	213[2]	231[2]
Total Protein, gm%	5.8	5.9	5.9	5.9	6.0	6.0
Albumin, gm%	2.6	2.8	2.7	2.4	2.8	3.0[2]
Globulin, gm%	3.2	3.1	3.2	3.4[2]	3.2	3.0[1]
A/G	0.8	0.9	0.9	0.7[1]	0.8	1.0[2]

[1] $p \leq 0.05$.

[2] $p \leq 0.01$.

TABLE 9

Summary of Clinical Chemistry Data For Male Osborne-Mendel Rats Receiving Chloroform In Their Drinking Water

18-Month Sacrifice

		Matched	Chloroform (ppm)			
Parameter	Control	Control	200	400	900	1800
Glucose, mg%	185	213	177	169	195	178
BUN, mg%	32	24	36	26	22^1	25
Creatinine, mg%	0.8	0.7	0.9	0.7^1	0.7^2	0.7
Uric Acid, mg%	1.9	2.9^1	2.1	2.0	2.2	2.2
Na^+, meq/l	148	148	148	149	148	148
K^+, meq/l	5.1	6.0	5.3	5.0	5.1	6.2^2
CO_2, meq/l	29	27	30	30	29	29
Cl^-, meq/l	102	102	100^1	101	102	104^1
Calcium, mg%	10.3	10.2	10.5	10.2	10.2	10.1
Phosphorus, mg%	5.2	5.5	5.2	4.9	5.3	6.4^2
Balance, Na-(Cl + CO_2)	17	19	18	18	17	15
Cholesterol, mg%	233	157^2	231	200	157^2	121^2
Triglycerides, mg%	375	225	373	255	130^2	41^2
Total Bilirubin, mg%	0.1	0.1	0.1	0.1	0.1	0.1
SGOT, mU/ml	99	153^2	106	99	122	92
SGPT, mU/ml	61	98^2	70	68	82	82^1
LDH, mU/ml	944	1005	1026	854	778	560
Alkaline Phosphatase, mU/ml	122	170^2	119	124	135	167^2
Total Iron, μg%	119	165^2	147^1	140^1	161^2	162^2
Total Protein, gm%	5.4	5.5	5.5	5.4	5.5	5.4
Albumin, gm%	2.2	2.3	2.2	2.2	2.5^2	2.6^2
Globulin, gm%	3.8	3.1	3.3	3.2	3.0^1	2.8^2
A/G	0.7	0.7	0.7	0.7	0.8^2	0.9^2

[1] $p \leq 0.05$.

[2] $p \leq 0.01$.

Table 10

SUMMARY OF CLINICAL CHEMISTRY DATA FOR MALE OSBORNE-MENDEL RATS RECEIVING CHLOROFORM IN THEIR DRINKING WATER

Two-Year Sacrifice

Parameter	Control	Matched Control	Chloroform (ppm) 200	400	900	1800
Glucose, mg%	147	151	147	141	154	143
BUN, mg%	46	25	32	45	22	27
Creatinine, mg%	1.2	0.7	1.0	0.9	0.8	0.7
Uric Acid, mg%	2.2	2.1	1.8	1.9	2.2	1.9
Na^+, meq/L	147	146	146	147	146	147
K^+, meq/L	5.6	6.3	5.4	5.8	5.4	5.9
CO_2, meq/L	30	29	31	30	30	31
Cl^-, meq/L	100	101	101	100	100	102
Calcium, mg%	10.7	10.1	10.4	10.5	10.4	10.3
Phosphorus, mg%	5.0	4.5	5.1	5.8	5.5	6.3
Balance, Na-(Cl + CO_2)	17	16	13	16	16	14
Cholesterol, mg%	252	135	213	217	152	121
Triglycerides, mg%	248	130	243	202	82	52
Total Bilirubin, mg%	0.1	0.1	0.1	0.1	0.1	0.1
SGOT, mU/ml	77	119	116	97	121	87
SGPT, mU/ml	51	77	103	77	89	68
LDH, mU/ml	493	590	580	571	786	450
Alkaline Phopshatase, mU/ml	101	114	87	104	113	122
Total Iron, μg%	112	172	133	154	170	177
Total Protein, gm%	5.2	5.5	5.3	5.4	5.8	56
Albumin, gm%	2.0	2.3	2.1	2.2	2.6	2.6
Globulin, gm%	3.2	3.2	3.2	3.3	3.3	2.9
A/G	0.6	0.8	0.7	0.7	0.8	0.9

PROCEEDINGS OF THE
SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Urinary Cancer-Related Protein EDC1 as Immunodiagnostic Marker in Breast Cancer

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ABSTRACT

A novel glycoprotein labeled EDCl, M_r27.5 K, was isolated originally from the urine of a leukemic patient E.D. This protein inhibited trypsin, chymotrypsin and human plasmin and was immunologically related to a normal plasma protein inter-α trypsin inhibitor (IATI) -M_r 160 K. EDCl was also the major component of a specific low molecular weight proteinuria encountered in most cancer patients.

By using a specific and sensitive radioimmunoassay for EDCl, urinary levels of this protein were measured in i) normal healthy women, ii) women with metastatic breast cancer, iii) patients with non-neoplastic diseases, and iv) preoperative patients with localized breast mass.

The urinary excretion of EDCl (mg/g creatinine) was classified in four ranges: i) normal <15 mg; ii) light 15-30 mg; iii) intermediate 31-45 mg; and iv) heavy >45 mg. The ave±SEM values were as follows: 8.0±2.2 for normal women and 98.6±11 for metastatic breast cancer patients. Sixty-six out of 82 patients with non-cancer diseases had an ave EDCl level of 14.6±4; the remaining had an ave of 94.8±16. The latter subgroup consisted of patients with renal failure, rheumatoid arthritis and certain infectious diseases. However, 60 to 90 percent of urinary immunoreactive EDCl (IR-EDCl) material in these diseases was of higher molecular weight (presumably plasma IATI) and its excretion was positively correlated with the degree of renal insufficiency in these patients. In the 26 preoperative patients, subsequently shown to have benign lesions, the EDCl excretion was 21.5±3; 24 of these patients were in the normal to light and 2 were in the intermediate range of excretion. In the other preoperative subgroup (27 patients subsequently shown to have malignant lesions), the ave EDCl excretion was 43.1±7 mg with 8 normal, 5 light, 4 intermediate and 10 heavy excre-

tors. A direct correlation existed between the number of nodes and the level of EDC1. Post-operative follow-up in heavy excretor preoperative patients showed a marked decline in EDC1 following the surgical removal of the tumor (ave 171 to 21 mg). Analyses of fractions obtained by gel-filtration on HPLC of plasma of metastatic breast cancer patients showed no significant accumulation of EDC1 in plasma, suggesting a rapid clearance of EDC1 by kidneys. Further analysis showed a negative correlation between plasma levels of IATI and urinary EDC1 levels in metastatic breast cancer patients.

In normal plasma, the IR-IATI material was found to exist in three molecular forms viz. with M_r 160 K, 120 K and 58 K. In patients with metastatic breast cancer, who were heavy excretors of EDC1, the IR-IATI corresponding to M_r 58 K was absent and their total IR-IATI was about 2/3 of that in normals. When normal plasma was incubated with trypsin or with human plasmin for 4 hours, the IR-IATI shifted to the M_r 30 K region. These data suggest that in cancer urinary EDC1 may arise as a result of selective degradation of IATI by cancer-associated proteolysis and that IATI may be one of the primary inhibitors for this enzymatic activity.

Introduction

In 1976, our laboratory reported the isolation of a novel glycoprotein, labeled EDC1, from the urine of a patient, E.D., with acute myelocytic leukemia. Its molecular weight was determined to be 27,500 by ultra-centrifugation and 32,000 by analysis on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). By a specific double antibody radioimmunoassay (RIA), the glycoprotein was detected in large quantities in urine of patients with different types of metastatic cancer (1). Subsequently, we showed that EDC1 accounted for 60-75% of the total protein excreted by patients with metastatic cancers (2).

EDC1 has been found to be immunologically related to a normal plasma proteinase inhibitor called inter-alpha trypsin inhibitor (IATI) and was also found to inhibit trypsin and chymotrypsin (3). Both IATI and EDC1 inhibited incorporation of thymidine by lymphocytes stimulated with phytohemagglutinin (PHA). This was explained on the basis that these molecules exerted this effect by inhibiting a critical endogenous proteinase induced by PHA on the surface of lymphocytes and required for blastogenesis (5).

In this report, we present data on the first detailed clinical trial of EDC1-proteinuria as an immunodiagnostic agent in a population of patients with adenocarcinoma of breast. We have determined the level of this protein in i) metastatic breast cancer patients, ii) age-and-sex matched healthy normal subjects, iii) non-cancer hospitalized patients, and iv) pre-operative patients, who initially presented with a localized breast mass.

Materials and Methods:

1. EDCl-isolation and purification This procedure has been published and was used with only slight modifications (1). Each batch of the glycoprotein was tested for its homogeneity on PAGE, SDS-PAGE, and by immunoelectrophoresis.

2. RIA for EDCl

The procedure for iodination of EDCl was as reported earlier (1). The homogeneity of iodionated EDCl was tested by PAGE and by gel-permeation chromatography. The antiserum to EDCl was raised in rabbits and tested for its specificity and titre as reported earlier (1).

The procedure for RIA of EDCl in urines and other samples was modified from our previously published technique (1). Aliquots of diluted antiserum (200 µl), diluant (500 µl of 1% ovalabumin in 0.05 M sodium phosphate buffered saline pH 7.0), and labeled EDCl (15,000 CPM in 100 µl) were incubated for 2 hours at 37° with constant shaking. Thereafter 100 µl aliquots containing 1-300 ng EDCl or diluted urines or plasmas were added and incubation was continued for 4 hours. The precipitation of antigen-antibody complex was achieved by adding 100 µl of appropriately diluted antibody (IgG fraction) to normal rabbit serum (raised in goat) and incubating 16 hours at 4°. The tubes were centrifuged (30 min: 6,000 g) and the radioactivity of supernatant fluid and precipitate was measured.

All urine specimens were analyzed at two dilutions - 1 in 10 and 1 in 100, while the plasma samples were analyzed at 1 in 50 and 1 in 500 dilutions. All samples were analyzed in duplicate.

3. Sephadex G-75 chromatography of urine of a patient with rheumatoid arthritis

A 5 ml aliquot of urine was chromatographed on a Sephadex G-75 column

(2.5 cm x 100 cm) which had been pre-calibrated as described previously (1). The column was eluted with 1 N acetic acid and 10 ml fractions were collected. Each fraction was analyzed for immunoreactive EDCl (IR-EDCl) as described under 2.

4. Gel permeation chromatography of plasma

a) High-performance liquid-chromatography (HPLC)

The procedure used was based on that of Regnier et al (6) and Rubenstein(7). The I-125 column of Waters was used; eluting buffer was 0.2 M phosphate buffer with 0.15 M sodium sulfate (pH 6.8). The column was calibrated using immunoglobulin, albumin, ovalbumin, chymotrypsinogen, and bacitracin. In a typical analysis, 150 µl of 1 in 10 diluted plasma was loaded on the column; the elution rate was 1 ml/min and 1 ml fractions were collected. Each fraction was analyzed for IR-EDCl as described under 2.

b) Ultrogel AcA34 chromatography

Three milliliters of plasma were fractionated on a column of Ultrogel AcA34 (2.6 cm x 100 cm) using 0.1 M $NaHCO_3$ buffer with 0.5 M NaCl (pH 8.3) as the eluting solvent. Each fraction (10 ml) was analyzed at two dilutions for its IR-EDCl contents by the procedure outlined earlier.

5. Incubation of plasma with Trypsin

One ml of normal plasma was mixed with equal volume of a trypsin solution in 1 mM HCl (1 mg/ml). The mixture was incubated at 37° and aliquots were withdrawn at 1 hour, 4 hour, and 6 hour intervals. The aliquots were mixed with 1 mM benzamidine solution and frozen till ready for analysis on HPLC. Following chromatography, the fractions were analyzed for IR-EDCl activity as described in 2 above.

Results

1. EDC1-levels in urine

The data on urinary EDC1 levels (expressed as mg/g urinary creatinine) of different groups are shown in Table 1.

The average (±SEM) excretion of EDC1 in 37 normal healthy subjects was 8.0±2.2, with most of the values below 10 mg/g creatinine; the average (±SEM) excretion of EDC1 in 40 metastatic breast cancer patients was 98.2±11.6.

For quantifying EDC1-proteinuria, the non-cancer patients were considered in two subgroup. The first subgroup consisted of patients with cardiovascular, pulmonary, and gastrointestinal diseases and other diseases such as lupus, dystrophy, neuralgia, and diabetes. The average excretion of EDC1 in these patients ranged from 3.2 to 14.3 mg/g creatinine-well within the normal range. The second subgroup consisted of patients with renal insufficiency, rheumatoid arthritis, and infectious diseases such as pneumonia and urinary tract infections. The overall IR-EDC1 levels in these patients were high- 75 to 200 mg/g creatinine. The urine specimens of these patients were chromatographed on a pre-calibrated Sephadex G-75 column and the fractions were analyzed for IR-EDC1 by the RIA procedure described earlier. The results of a chromatographic analysis of urine of a rheumatoid arthritic patient are shown is figure 1. More than 70 percent of IR-EDC1 eluted in the void volume (M_r>70,000). For patients with renal insufficiency or with pneumonia, 90-95% of immunoreactive material was of M_r> 70,000.

2. Plasma EDC1 levels

In order to determine the blood levels of EDC1 it was important to remove the cross-reacting IATI (M_r 160,000) from the plasma. This was done by gel permeation chromatography of diluted plasma and by analyzing each fraction for its IR-EDC1 level. The results of HPLC analyses are shown in figure 2. In normal healthy subjects, two peaks of immunoreactivity were usually noted-one in the void volume, M_r>100,000, and the other at M_r 55,000. The second peak was broader (2-3 fractions) in some cases although its elution time remained essentially unchanged. This peak also accounted for almost one-third of the total IR-EDC1 in plasma and was absent in plasma of most patients with metastatic breast cancer who were also heavy excretors of EDC1. No significant accumulation of EDC1 was observed (in the molecular weight range of 30,000) in any metastatic breast cancer plasma.

The plasma obtained from analysis of normal plasma by an Ultrogel AcA34 column chormatography are shown in figure 3. In most normal plasma, three immunoreactive peaks of EDC1 were identified-the first at M_r 160,000, the second at M_r 120,000 (shoulder) and the third at M_r 58,000. No significant amount of IR-EDC1 could be detected in the M_r range of about 30,000 in normals or in metastatic breast cancer patients. The IR-EDC1 peak of M_r 58,000 was invariably absent in breast cancer patients who were also heavy excretors of EDC1.

The effect of trypsinolysis (4 hr) of normal plasma on the pattern of immunoreactivity is shown in figure 4. Treatment with trypsin apparently does not effect the chromatographic pattern (A_{280}) but does cause an increase of IR-IATI in the lower molecular weight range with a simultaneous decrease in immunoreactive peak in the void volume (M_r>100,000).

Discussion

In 1979, we reviewed the prevalence of low molecular weight proteinuria in patients with different types of metastatic cancers (2). Of the five urinary cancer-related glycoproteins identified in our laboratories, EDC1 accounted for 50-80 percent of the total urinary proteins in the cancer patients. This urinary factor was also found to be a compettive inhibitor of trypsin and immunologically related to a normal plasma protein IATI. In the present study, we have investigated the immunodiagnostic applications of EDC1-proteinuria in adenocarcinoma of the breast, with special emphasis on the following questions:

a) What are the urinary and plasma levels of EDC1 in patients with metastatic breast cancer and in age-and-sex matched healthy normal subjects?

b) Is elevated level of EDC1 in plasma and/or urine cancer specific?

c) Does either value distinguish between preoperative benign and malignant lesions? And

d) Does either value predict postoperatively which patients are "cured" and which patients will develop a recurrence?

This work shows that there is no significant accumulation of EDC1 in the plasma of normal or metastatic breast cancer patients (Cp HPLC profiles in figure 2.) However, from the data shown in Table 1, it is clear that EDC1 is a minor component of normal urine and its excretion in metastatic breast cancer is significantly elevated ($p<.001$). Heavy excretion of IR-EDC1 was noted in the non-cancer group of patients with renal insufficiency, rheumatoid arthritis, and certain infectious diseases. This source or false positives was eliminated when the urine was appropriately pre-treated to remove the interferring high molecular weight material (presumably plasma IATI).

Among the 25 preoperative patients with malignant tumors, 12 excreted more than 30 mg EDC1/g creatinine and of these 8 were distinctly heavy excretors. The urinary output of EDC1 in these patients showed a marked drop following a surgical removal of the tumor. These statistics suggest that EDC1-proteinuria is a reliable tool to monitor the patient's response to the prescribed therapy. At this stage, we have no data to verify whether or not this immunodiagnostic agent can be used to predict a recurrance of the disease. None of our patients in the long term study have so far shown elevated EDC1 excretion or had a relapse (Table 2).

The depressed systemic levels of IATI and elevated urinary levels of EDC1 in patients with metastatic breast cancer provide an insight into the role of the plasma antiproteinase in cancer. There is ample evidence in literature to show the enhanced proteolytic activity in the malignant breast tumor. For example, Recklies et al have shown that breast tumor in organ culture secretes collagenase, cathepsin B, cathepsin G and plasminogen activator (9,10). IATI may bindand inhibit one or more of these enzymes and in the process be degraded to EDC1, which in turn may be rapidly cleared by kidney. The formation of EDC1 like molecule on trypsinolysis of normal plasma (Figure 4) further support the above postulate.

The physiologic significance of three mass variants of systemic

IATI (Figure 3) identified in the present study is not understood at present. Further work on the biologic function of IATI is in progress in our laboratories and may provide a clue on the role of three forms of plasma IATI.

Table 1. Urinary excretion of EDC1 in clinical population

Disease		Urinary EDC1 (mg/g creatinine) (mg/g creatinine) ave ± SEM	<15 mg (normal)	15-30 mg (light)	31-45 mg (intermediate)	>45 (heavy)
I.	Normal healthy controls	8.0 ± 2.2	35	2	0	0
II.	Metastatic breast cancer	98.2 ± 11.6	4	5	10	21
III.	Non-cancer diseases					
	1. Cardiovascular diseases	3.2 ± 1.3	16	0	0	0
	2. Pulmonary diseases	14.3 ± 6.0	5	1	0	0
	3. Gastrointestinal diseases	12.6 ± 3.2	7	2	1	0
	4. Other diseases (e.g., lupus, diabetes, dystrophy and neuralgia)	7.3 ± 1.2	23	8	0	0
	5. Renal insufficiency	192.8 ± 81.1 (>90% M_r ~160,000)	0	0	1	3
	6. Rheumatoid Arthritis	74.8 ± 23.5 (>65% M_r ~160,000)	2	2	1	5
	7. Infectious diseases	132.6 ± 26.4 (>90% M_r ~160,000)	0	0	0	5
IV.	Preoperative patients					
	1. Benign lesions	21.5 ± 3.4	12	12	2	0
	2. Malignant lesions	43.1 ± 7.6	8	5	4	8

TABLE 2. URINARY IR-EDC1 LEVELS OF PREOPERATIVE PATIENTS (EXPRESSED AS MG/G CREATININE).

	Preoperative	Postoperative					
Patient #		1st Followup	2nd Followup	3rd Followup	4th Followup	5th Followup	6th Followup
301	130	7.0	14.7	15			
304	61	12.0	8.0	7.7	16	27	
305	67	10.4	24.5		22		
306	136	76.0	7.2	12.9	25		
307	45	13	8.4	6	4		40
310	410	7	17	18	5.2		
318	42	10.6	11.3	13	11		
319	285	2.4		44	24	33	18
321	72	6.3	11	34	25		
327	182	26.3	14				

Legends to Figures

Figure 1: A chromatographic profile of 5 ml of urine from a patient with rheumatoid arthritis on a Sephadex G-75 column (2.5 x 100 cm). The eluant was 1 N acetic acid and 10 ml fractions were collected. Each fraction was analyzed for IR-EDCl activity and results are shown in solid line. Horizontal axis shows elution volume and vertical axis shows A_{280} and IR-EDCl (ng/ml).

Figure 2: High performance liquid chromatography (HPLC) analysis of an aliquot (150 µl) plasma, diluted in 10, on Waters I-125 gel permeation column horizontal axis, elution time in minutes. On vertical axis, the solid line represents A_{280} and the broken line represents the profile of IR-EDCl activity. Eluting solvent was 0.2 M sodium phosphate with 0.15 M sodium sulfate (pH 6.8). The column was calibrated by eluding a mixture of standards: immunoglobulin (elution time 10 min.), albumin (14 min.); ovalbumin (17.5 min.), chymotrypsinogen (19 min.) and bacitracin (22 min.). The flow rate was 1 ml/min and fraction size was 1 ml.

Figure 3: Chromatographic profile of 3 ml normal plasma on an Ultrogel AcA34 column (2.6 x 100 cm). The column was calibrated by eluding a mixture of blue dextran, IgG, albumin, ovalbumin, chymotrypsinogen, and ribonuclease. Flow rate was 0.5 ml/min and the fraction size was 10 ml.

Figure 4: HPLC analysis of an aliquot (150 µl) of normal trypsinized plasma diluted 1 in 10. The plasma had been incubated with trypsin at 37° for 4 hr. Other conditions were the same as in Figure 2.

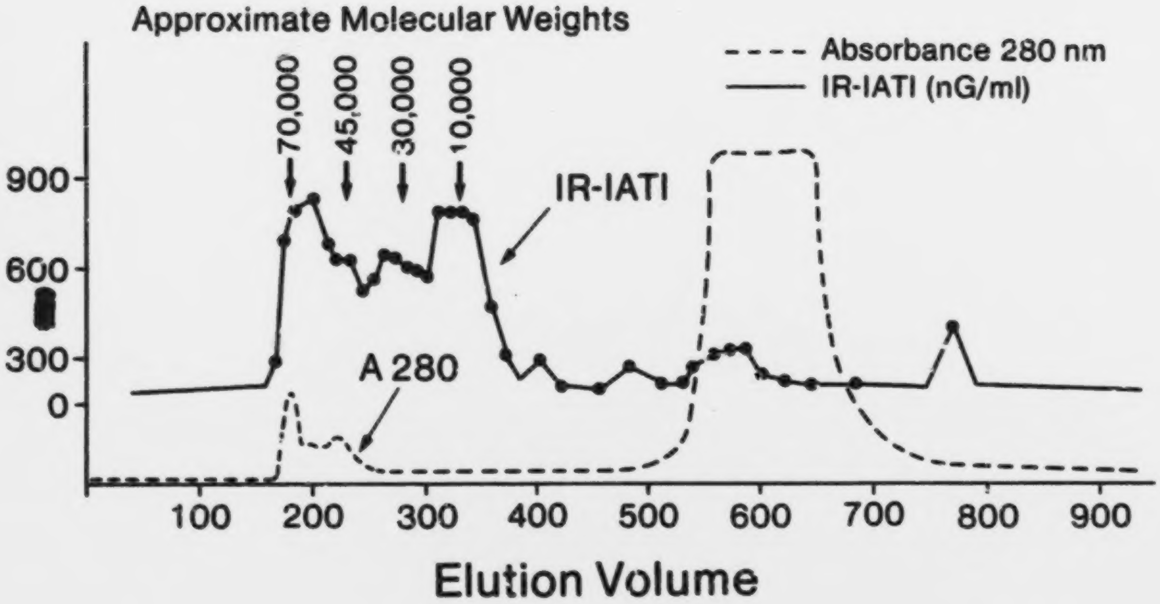
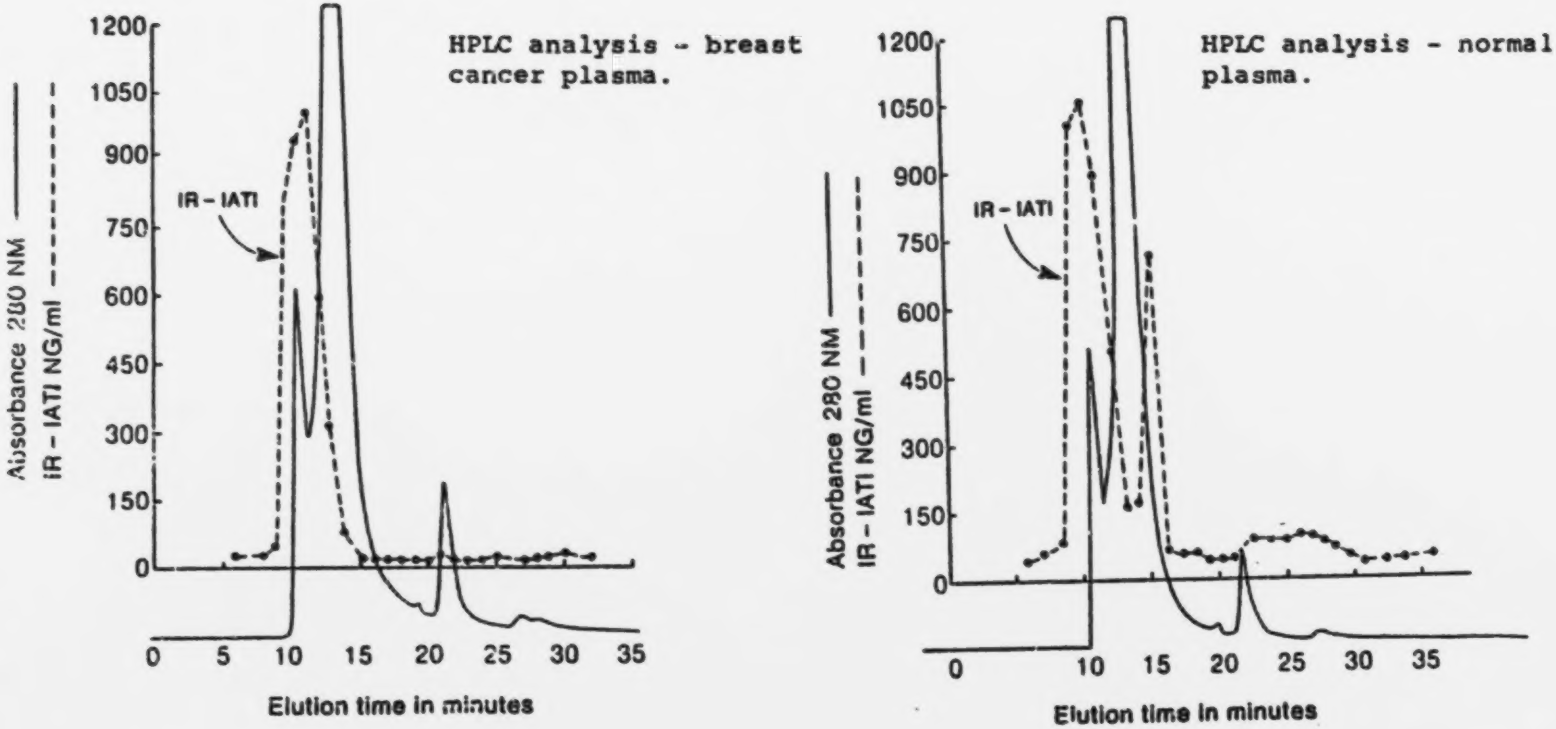

Approximate Molecular Weights
70,000
45,000
30,000
10,000
- - - - Absorbance 280 nm
—— IR-IATI (nG/ml)
IR-IATI
A 280
900
600
300
0
100
200
300
400
500
600
700
800
900
Elution Volume

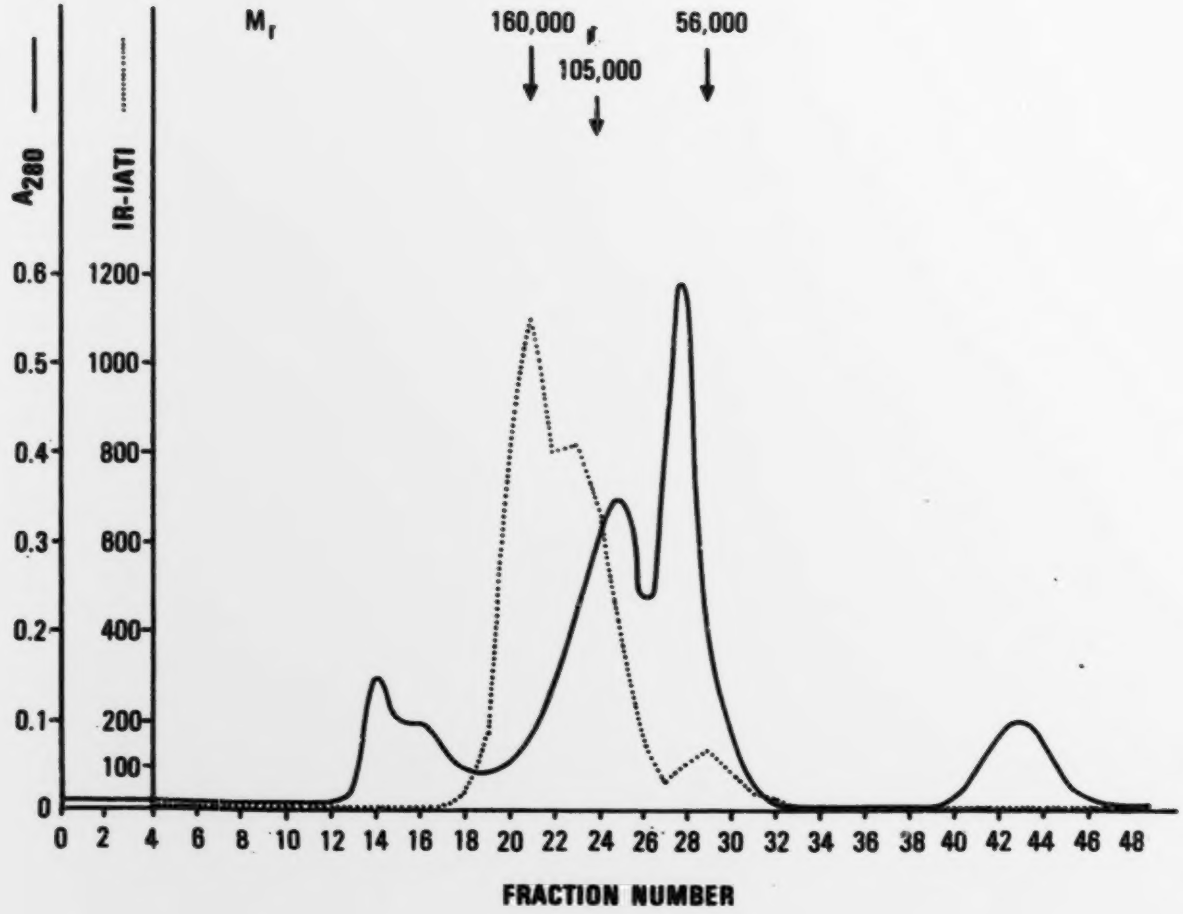
Figure 1


HPLC analysis - breast cancer plasma.

HPLC analysis - normal plasma.

Figure 2

617



A280
IR-IATI
Mr
160,000
105,000
56,000
0.6
0.5
0.4
0.3
0.2
0.1
0
1200
1000
800
600
400
200
100
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48
FRACTION NUMBER

618

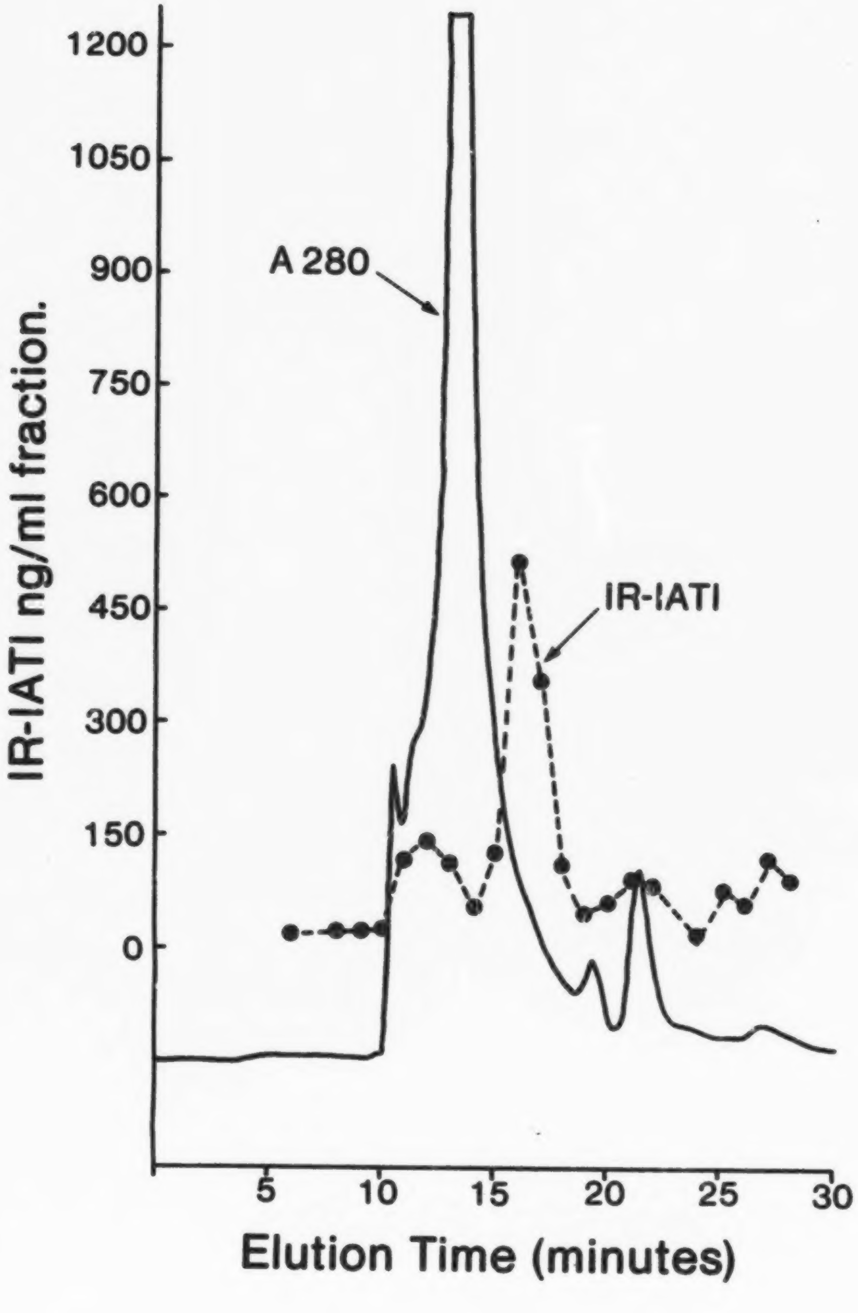

IR-IATI ng/ml fraction.
1200
1050
900
750
600
450
300
150
0
A 280
IR-IATI
5
10
15
20
25
30
Elution Time (minutes)

Figure 4

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MS. CORRACUA: I would like to know if you followed also the output in pregnant ladies.

DR. CHAWLA: In pregnant urine we find the detectable level of this protein but not in that high a level. We find anywhere from 15 to 30 milligrams per gram creatinine. But it is not as high as in leukemia or in metastatic breast cancer.

SPEAKER: Dr. Chawla, I am just wondering if you had any idea of any similar studies that might be going on in animal systems, looking for marker proteins in the urine?

DR. CHAWLA: I don't think I am aware in the literature. I have been confining myself to IATI and I do notice that the rabbit is supposed to have a protein that cross reacts to human IATI, and some of these fragments have been reported in some tumor urine but I am not sure how similar or dissimilar it is to EDC1.

SPEAKER: I have another question, a brief one. Have you looked at any combinations or batteries of tests -- a few years back we were looking at CEA and we were also looking at HCG in combination in breast cancer. Have you looked at this with any of these?

DR. CHAWLA: That is one of the things that we plan to do in the remaining life of this contract. But we did include some of these patients -- we had an earlier report on use of cyclic GMP and cyclic NPS tumor markers and we found that cyclic GMP had a pretty good correlation with these values that we were getting on metastatic breast cancer with EDC1.

DR. MORRIS. Thank you.

PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Analysis of Normal and Malignant Lymphoid Progenitor Cells with Monoclonal Antibodies

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ANALYSIS OF NORMAL AND MALIGNANT LYMPHOID PROGENITOR CELLS WITH MONOCLONAL ANTIBODIES

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ABSTRACT

A major objective of our laboratory has been to study the cellular and molecular events associated with the differentiation of normal and malignant human lymphoid progenitor cells. To this end, we have produced a panel of monoclonal antibodies (designated BA-1, BA-2, and BA-3) against the human pre-B acute lymphoblastic leukemia cell line NALM-6-M1. Although some overlap exists in the reactivity of these antibodies with a variety of normal and malignant lymphoid cells, each antibody identifies a distinct cell surface structure. All three antibodies appear to bind primarily (but not exclusively) to cells at some stage of B lymphocyte development. A major effort is currently underway to use these antibodies in: a) the immunologic classification of leukemia/lymphoma, and b) as probes for identifying lymphoid progenitor cells in human bone marrow and thymus.

INTRODUCTION

In man most tissues consist primarily of differentiated cells that normally show little evidence of proliferation and self-renewal. Exceptions to this are the gastrointestional tract, skin, and hematopoietic tissues such as BM. In these tissues a small number of stem cells have the capacity for self-renewal and differentiation, and the more mature cells are derived from these stem cells. Various stem cell abnormalities often lead to serious illness, and many diseases of the hematopoietic system appear to be stem cell disorders. Such diseases include aplastic anemia, acute and chronic myelocytic leukemia, and acute lymphoblastic leukemia.

Acute lymphoblastic leukemia (ALL) is a complex disease of unknown etiology. Generally considered to involve cells early in lymphoid development (1), ALL exhibits substantial biological and clinical heterogeneity (2). Studies in a number of laboratories over the past several years have indicated that immunologic markers, particularly those identifying cell surface structures, may be highly discriminating in the identification of various malignant and normal cellular components of the hematopoietic system. Antisera developed against leukemia-associated antigens and differentiation antigens of the T and B lymphocyte lineages have become increasingly important in a) the classification and diagnosis of ALL(2,3) and b) the analysis of normal lymphoid counterparts in BM and thymus (4). With the advent of monoclonal antibody production (5) has come the hope that a definitive and complete analysis of cell surface structures can be realistically accomplished in the future.

Our laboratory has recently produced a panel of monoclonal antibodies (designated BA-1, BA-2, and BA-3), against the human pre-B acute lymphoblastic leukemia cell line NALM-6-M1 (6,7). In this brief report we will summarize our experience to date with these antibodies. The reader is referred to several other publications for a more thorough overview of monoclonal antibodies recognizing antigens on human lymphoid cells (8-10).

MATERIALS AND METHODS

The methods used in the production, characterization, and utilization of all our monoclonal antibodies can be found in published reports (6,7,11).

RESULTS AND DISCUSSION

Monoclonal Antibody BA-1

Table 1 summarizes some of the more salient characteristics of monoclonal antibody BA-1. This antibody binds primarily to cells in the B lymphocyte lineage, including all surface Ig$^+$ cells in blood and bone marrow. The antigen recognized by BA-1 is lost as B cells terminally differentiate into plasma cells. BA-1 also binds weakly to mature granulocytes and some myeloid precursors, including myelocytes (12). Extensive examination of normal bone marrow has revealed that the percentage of BA-1$^+$ cells decreases with age, and that approximately 50% of bone marrow TdT$^+$ cells express the BA-1 antigen (C.J. Brashem, M.S. thesis, University of Minnesota, 1981).

Cells from over 200 leukemic patients have been evaluated with BA-2. The majority (∿80%), of non-T ALL, 90% of surface Ig$^+$ non-Hodgkins lymphomas, and >95% of surface Ig$^+$ chronic lymphocytic leukemias are reactive with BA-1. Weak binding occurs with a small minority (<20%) of AML and T cell ALL are uniformly negative.

Functional studies have shown that BA-1 does not bind to NK cells. Extensive studies of human bone marrow indicate that treatment with BA-1 and complement does not appreciably suppress the growth of several hematopoietic stem cells, including CFU-GEMM, CFU-C, CFU-E, and BFU-E (12). This absence of reactivity with hematopoietic stem cells, coupled with the fact that most (>80%) non-T ALL are BA-1$^+$, makes this antibody an excellent candidate for the in vitro removal of residual leukemic cells prior to autologous bone marrow transplantation.

Monoclonal Antibody BA-2

Table 2 summarizes the characteristics of monoclonal antibody BA-2. This antibody binds to a small percentage of lymphoid cells in bone marrow, thymus, and peripheral blood. The predominant phenotype of the BA-2$^+$ cell in bone marrow and peripheral blood is E rosette$^-$, surface Ig$^-$, nonphagocytic, i.e., most BA-2$^+$ cells are probably lymphoid precursors. Like BA-1, examination of normal bone marrow has revealed that the percentage of BA-2$^+$ cells decreases with age. Approximately 60% of bone marrow TdT$^+$ cells are BA-2$^+$ (8,13).

Cells from over 200 leukemic patients have also been evaluated with BA-2. BA-2 binds strongly to ∿70% of ALL (including most pre-B), and weakly to ∿20% T-cell ALL, and 25% of AML. BA-2 also shows strong reactivity with a large number of nonhematopoietic tumors including melanomas and neuroblastomas.

Functional studies have shown that addition of BA-2 and complement to normal bone marrow does not inhibit the growth of CFU-GEMM, CFU-C, CFU-E, and BFU-E (Ash et al. Manuscript in preparation). Like BA-1, this makes BA-2 an ideal candidate for the in vitro removal of residual leukemic cells prior to autologous bone marrow transplantation.

BA-2 precipitates a 24,000 dalton (p24) cell surface structure under reducing or nonreducing conditions. Recent experiments have shown that p24 is not an integral membrane protein, and that the polypeptide chain may contain small amounts of carbohydrate (14).

In an attempt to develop a model for the differentiation of lymphoid progenitor cells, we have recently cultured a variety of ALL cells with the phorbol ester, TPA. As reported elsewhere (15,16) we have successfully induced the expression of p24 in a number of non-T, non-B ALL. These preliminary observations are encouraging and studies are continuing in this area.

Monoclonal Antibody BA-3

We have recently produced a third monoclonal antibody, designated BA-3. As summarized in Table 3, this antibody is serologically identical to J-5, a monoclonal antibody identifying the common ALL antigen (17). We are currently working on determining whether BA-3 precipitates the same 100,000 dalton cell surface glycoprotein precipitated by J-5 (17).

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TABLE 1

MONOCLONAL ANTIBODY BA-1

1. Specificity for normal cells: Binds strongly to all SIg^+ cells and a small number of SIg^- lymphoid cells in blood and bone marrow (some $BA\text{-}1^+$ cells are TdT^+). Binds weakly to granulocytes and some myeloid precursors including myelocytes. No binding to thymocytes, T cells, plasma cells, monocytes, platelets or RBC.

2. Specificity for malignant cells: Binds strongly to ∿80% of non-T ALL (including all pre-B), ∿90% SIg^+ non-Hodgkin's lymphomas, and all SIg^+ CLL. Binds weakly to some AML. No binding to T-cell leukemia/lymphoma, myeloma, and most AML.

3. BA-1 does not bind to natural killer (NK) cells. BA-1 does not appreciably bind to several hematopoietic stem cells, including CFU-GEMM, CFU-C, CFU-E, and BFU-E.

4. The cell surface determinant recongized by BA-1 is not surface Ig, HLA-DR, or receptors for C3 or Fc. Preliminary evidence in SDS-PAGE suggests that BA-1 precipitates a two chain component of 27,000 and 55,000 daltons.

TABLE 2

MONOCLONAL ANTIBODY BA-2

1. Specificity for normal cells: Binds strongly to 5-10% of E^-, SIg^- bone marrow lymphoid cells (some being TdT^+), $\sim$8% of thymocytes, and $\sim$3% of peripheral blood lymphocytes. Also binds to platelets and nonhematopoietic cells from various human tissues.

2. Specificity for malignant cells: Binds strongly to $\sim$70% of ALL (including most pre-B). Binds weakly to $\sim$50% SIg^+ CLL, <20% T-cell leukemia/lymphoma, and AML. Also binds to many nonhematopoietic tumor cells.

3. BA-2 does not bind to several hematopoietic stem cells including CFU-GEMM, CFU-C, CFU-E, and BFU-E.

4. BA-2 precipitates a 24,000 dalton protein (p24) in SDS-PAGE under reducing or nonreducing conditions.

5. Induction studies with the phorbol ester TPA indicates that $p24^+$ leukemic, lymphoid cells can be generated from $p24^-$ precursors.

TABLE 3

MONOCLONAL ANTIBODY BA-3

1. Specificity for normal cells: Does not bind to peripheral blood mononuclear cells. Does bind to ∿1-2% of bone marrow lymphoid cells from pediatric donors.

2. Specificity for malignant cells: Binds strongly to ∿75% of non-T, non-B ALL and weakly to 10-20% of T-cell ALL. No binding to AML or CLL.

3. BA-3 is serologically identical to monoclonal antibody J-5 (anti-common ALL antigen).

PROCEEDINGS OF THE
SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

Friday Afternoon, September 11

METHODOLOGY, EXPERIMENTAL, AND MODELS SESSION - continued

Session Chairperson:
Dr. Umberto Saffiotti
National Cancer Institute

AFTERNOON SESSION

DR. SAFFIOTTI: Good afternoon. Congratulations for your enthusiasm for science. The afternoon session continues the session on methodology, experimental and models session. The first paper is going to be presented jointly by Dr. Leonard Schechtman from Microbiological Associates and Dr. Andrew Sivak from A.D. Little. Dr. Sivak is going to begin the presentation and then pass the microphone to Dr. Schechtman, and then somehow share the conclusions.

PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Evaluation of Transformation Assays Using C3H 10T1/2 Cells for Use in Screening

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EVALUATION OF THE TRANSFORMATION ASSAY USING C3H 10T 1/2 CELLS FOR USE IN SCREENING CHEMICALS FOR CARCINOGENIC POTENTIAL

by

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A. BACKGROUND

This report is part of the discussion about different methodologies that are of use in determining the potential health effects of chemicals and complex mixtures to which humans might be exposed.

In attempting to ascertain prospectively whether there are chemical agents that present a human biological risk, a battery of toxicological tests has been developed, ranging from chronic animal studies using various exposure methodologies, to the newly developed short-term tests. The short-term tests can be generally segregated into two categories, i.e. those that use mutational or other genetically related markers as an endpoint, and the neoplastic transformation assays, which use markers that appear to be more directly related to the entire carcinogenic process than to mutation per se.

An important reason for exploring the transformation assays is that there are some important qualitative differences between mutagenesis and carcinogenesis. Carcinogenesis is a multi-step developmental process that is influenced by a number of modifying factors. In contrast, somatic mutation is generally characterized as a single biological step which most often results in a detectable mutagenic endpoint.

The neoplastic transformation assays comprise a group of procedures that can be generally divided into three overall classes (Table 1). One type employs cell strains, which are early passage diploid cells; another uses cell lines which are established cell populations that are generally not diploid and generally not "normal," but they do have some appropriate properties that can be utilized for neoplastic transformation assays; a third set of assay procedures uses mammalian cells in conjunction with various viruses to detect chemical carcinogens.

This presentation will describe validation studies with a cell line designated C3H 10T 1/2 and the different methodological approaches independently employed in two different laboratories, i.e. Arthur D. Little, Inc. and Microbiological Associates. The C3H 10T 1/2 line was derived by Reznikoff and Heidelberger and their associates (1,2) nearly a decade ago, and there has been a considerable amount of work done with it in many laboratories. The benefits of using cell lines such as C3H 10T 1/2 include (1) availability of stable target cell populations, (2) a readily identifiable endpoint with minimal subjectivity used for the determination of transformation response, and (3) protocols subject to modification and modulation to increase the sensitivity of the assay and the breadth of compounds that can be detected.

Table 1

Cell Culture Neoplastic Transformation Assays

CELL STRAINS

Syrian hamster embryo (clonal)

Syrian Hamster embryo (focus)

Human fibroblast (focus)

CELL LINES

Mouse C3H-10T 1/2

Mouse BALB/c-3T3

Mouse prostrate fibroblast

Syrian hamster BHK-21

CELLS - VIRUS

Fischer 344 rat embryo cells + Rauscher leukemia virus

Syrian hamster + SA7 - Simian Adenovirus

B. COLLABORATIVE STUDY RESULTS OF ARTHUR D. LITTLE, INC., AND MICROBIOLOGICAL ASSOCIATES

The basic protocol of the C3H 10T 1/2 transformation assay currently being used at Arthur D. Little, Inc. and Microbiological Associates, is shown in Figure 1. It is essentially unchanged from the original protocol that was described by Reznikoff et al (2). One difference in the protocols used at Arthur D. Little, Inc., and Microbiological Associates is that the target cell population seeded per test plate is 10^4 and 10^3, respectively. This appears not to be a significant factor in the final outcome of the assay (data not shown).

Figure 2 shows the characteristic properties of a Type III focus which is the primary morphological endpoint of the assay. The Type III focus has been defined as a group of cells that form a multilayer, that have an intertwining random orientation at the periphery of the focus, and that grow out into the surrounding monolayer of normal cells. A Type II focus is one that also exhibits a multilayer growth pattern although the extent of growth into the monolayer is less pronounced than in Type III foci. Thus, the basic differences between the Type II and Type III phenotypes is the degree of morphological aberration associated with the focus.

The ability to distinguish between Types II and III foci does require some training, as does any morphological discrimination; however, with a modest amount of experience and collaboration with investigators skilled in performing the assay, the distinction can be made by those with training in the art of cell culture.

With respect to the basic behavior of the C3H 10T 1/2 cell line in the two laboratories engaged in the collaborative program with the National Cancer Institute, the cloning efficiency ranges are almost identical, and the saturation density is somewhat higher at Microbological Associates, however, this does not appear to be a major factor (Table 2).

Figure 3 shows important data demonstrating that in two separate laboratories, in assays done at different times, 3-methylcholanthrene (MCA) produced a positive dose-response with respect to Type III foci and the sum of Type II and Type III foci. The difference in responses between the two laboratories is small and well within bounds of experimental variability observed in this assay.

One of the key components in validating an assay system is to examine its responsiveness to a set of known model compounds. The data on some of these compounds is shown in Table 3. The three aromatic hydrocarbons that are known carcinogens were positive, however, dibenz(a,h)anthracene was marginal. Dibenz-

Table 2

Characteristics of C3H 10T 1/2 Cells in Collaborating Laboratories

	ADL	MA
Cloning Efficiency (%)	15 - 29	12 - 30
Saturation Density (x10^5/60 mm dish)	4.9 - 8.5	8.0 - 11.0

Note: Cloning experiments were performed by seeding 200 cells in 60 mm dishes and counting stained colonies 10-14 days later. Saturation density experiments were carried out in 60 mm Falcon dishes by determining the cell counts in confluent monolayer plates.

Table 3

Transformation Responses to Known Carcinogens and Non-Carcinogens in Collaborating Laboratories

CHEMICAL	ADL	MA
3-methylcholanthrene	+	+
7,12-dimethylbenz(a)anthracene	+	+
benzo(a)pyrene	+	+
dibenz(a,h)anthracene		± (+[b])
phenanthrene	-	- (-[b])
anthracene	-	- (-[b])
N-methyl-N'-nitro-N-nitrosoguanidine	± (+[a])	- (+[b])
ethylmethanesulfonate	+	+ (+[b])
N-acetoxy-2-fluorenylacetamide	± (+[a])	N.D.[c]
2-fluorenylacetamide	-	- (+[b,d])
2-aminoanthracene	-	+[d]

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[a] Cell cycle and/or cell density dependent
[b] Level II transformation
[c] N.D. Not done
[d] Exogenous metabolic activation required

(a,h)anthracene has extremely poor solubility in aqueous systems and this may be one reason why there is only a marginal response. Phenanthrene and anthracene were not active in vitro, and they are inactive in vivo as carcinogens.

Two other classes of chemicals on the validation list warrant further consideration. One class includes direct-acting agents that are very potent, such as N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). As was reported earlier from Heidelberger's group and confirmed in our laboratories, this chemical results in a very poor transformation response using the standard 10T 1/2 assay. It appears that the assay for these kinds of agents is cell cycle sensitive or requires modification for focus expression, and data will be presented below addressing this issue.

The other classes of compounds examined for which the standard assay appears not efficacious include those that require metabolic activation such as 2-fluorenylacetamide, 2-anthramine and nitrosamines. Although C3H 10T 1/2 cells are capable of metabolizing polycyclic aromatic, hydrocarbons, they apparently do not metabolize aromatic amines, nitrosamines and other diverse chemicals that generally require metabolic conversion before transformation or genotoxic activity can be demonstrated. For such agents, a metabolic supplement must be supplied to the C3H 10T 1/2 test system in order to render them biologically active. This issue is addressed in detail in this report.

C. INDEPENDENT RESEULTS OF ARTHUR D. LITTLE, INC.

1. Cell cycle requirements

In connection with the cell cycle sensitivity of the transformation assay when dealing with certain direct-acting agents, Figure 4 presents some relevant data. These studies with MNNG show that as cells enter and transverse through the S-phase of the cell cycle, as indicated by the DNA synthesis profile, the toxicity of MNNG increases. Thus, the toxicity of such direct-acting agents, particularly those with a short half-life in aqueous media, is dependent on these materials being active during DNA synthesis. Similarly, the transformation response is also sensitive to cell cycle in confirmation of previous reports. Other data presented later offer alternative suggestions as to the possible mechanism associated with the 10T 1/2 cell response to direct-acting alkylating agents.

2. Cell density studies

It appears, then from these studies that for certain classes of compounds a standard assay may not be appropriate. One alternative under investigation is to use increased target cell densities for the assay so as to put more cells at risk to chemical treatment. The data shown in Table 4 demonstrates that

Table 4

Cell Density Dependence of Transformation by Various Carcinogens

		PLATING DENSITY		
CHEMICAL	CONCENTRATION	10^3	10^4	10^5
		Type III foci/dish		
3-methylcholanthrene	5.0 μg/ml	0.09	0.93	0.00
		0.54	1.34	--
dibenz(a,h)anthracene	100.0 μg/ml	0.00	--	--
		0.10	0.00	0.00
N-methyl-N'-nitro-N-nitrosoguanidine	1.0 μg/ml	0.00	--	--
		0.06	--	--
		--	0.00	0.36
		--	0.28	0.36
N-acetoxy-2-fluorenylacetamide	1.0 μg/ml	0.00	--	--
		0.00	--	--
		--	0.00	0.00
	2.5 μg/ml	--	0.00	0.06
	5.0 μg/ml	--	0.00	0.07

direct-acting agents, not readily identified as having transformation potency using the standard assay, do show activity at higher cell densities (e.g. $1 \times 10^4 - 1 \times 10^5$ cells/dish) than normally used (1×10^3 cells/dish). This positive response is most likely due to an increased number of cells randomly entering the sensitive S-phase of the cell cycle.

3. Hepatocyte-mediated metabolic activation

As was indicated above, C3H 10T 1/2 cells apparently do not have the capability to metabolize a wide range of chemical classes, other than polycyclic aromatic hydrocarbons. Therefore an alternative means of supplying an exogenous form of metabolic activation using rat hepatocytes was examined. Figure 5 shows that primary rat hepatocytes are far superior to 10T 1/2 cells themselves in metabolizing 3-methylcholanthrene to water soluble metabolites. It is anticipated that the hepatocytes would metabolize most xenobiotics well based on these results and those from other laboratories.

In attempting to evaluate the biological response of C3H 10T 1/2 cells to chemical carcinogens in the presence of primary hepatocytes, a cocultivation procedure has been employed by plating a dense culture of C3H 10T 1/2 cells and overlaying this culture 24 hours later with a dense population of rat hepatocytes. As can be seen from Figure 6, the hepatocytes attach to the C3H 10T 1/2 cell monolayer, which remains readily discernable in the coculture. Also shown is a culture of C3H 10T 1/2 target cells alone and a culture of target cells and hepatocytes treated with cyclophosphamide. In the treated mixture culture, the lower layer of the target cells has almost disappeared, indicating that the hepatocytes have converted cyclophsophamide to a highly cytotoxic agent that results in severe death of the target cells. In view of such promising results, efforts are now in progress to evaluate the feasibility of hepatocyte-mediated C3H 10T 1/2 cell transformation by chemical carcinogens. Current efforts involve establishing a better understanding of the methodologies associated with a mixed hepatocyte-fibroblast cell culture. One of the problem areas, for example, is the difficulty of quantitation of the different cell types (target 10T 1/2 vs. hepatocytes) where morphological criteria are not feasible. Under assay conditions in which it is necessary to replate target cells from treated cocultures, this may be of significant importance.

4. In vivo tumorigenicity studies

Beyond the morphological analysis of transformed foci, the determination of whether or not these foci are, indeed, tumorigenic in vivo is a key component to the overall validation of the assay. Although early results in other laboratories indicated that Type III foci often gave rise to tumors in appropriate hosts, recent experience at Arthur D. Little, Inc. has shown that this occurrence of Type III foci giving rise to tumors is not as regular or as frequent as one might have expected from earlier studies.

Table 5 shows some experiments in this area where a large series of Type III foci taken from the same experiment were passaged and analyzed in parallel. As can be seen, relatively few of these foci grew in agarose in early passage and still fewer grew as tumors in vivo. With increasing numbers of cell passage the likelihood increases for growth in semi-solid medium or in vivo tumorigenicity. This progressive acquisition of properties associated with the transformed phenotype is a recognized phenonemon in all in vitro transformation systems. However, there appear to be some discontinuities in the tumorigenicity of morphologically transformed 10T 1/2 cells in vivo. From preliminary experiments, some in our laboratory and other studies reported in the literature, host factors appear to be extremely important. Primary among the factors is the host animal one chooses (e.g. newborn C3H, irradiated weanling C3H, athymic, etc.) (Table 6). These observations support the view that the final validation of the ability of a focus to form a tumor in vivo is subject to a considerable number of both intrinsic and extrinsic variables including immunological status of both host and transformed cells, as well as the relationship between phenotypic progression, tumor frequency and the number of cell passages.

D. INDEPENDENT RESULTS OF MICROBIOLOGICAL ASSOCIATES

As illustrated in the foregoing discussion, the C3H 10T 1/2 cell system has a variety of attributes, but appears to require assay modifications in order to make it applicable as a generalized screening procedure. To this end, efforts at Microbiological Associates have concentrated on methods of enhancing the sensitivity of 10T 1/2 cells to diverse classes of chemical carcinogens. These include a modification of the standard ("Level I") transformation assay designated the C3H 10T 1/2 Cell Amplification ("Level II") Transformation assay, and the incorporation of an exogenous sub-cellular tissue homogenate as a metabolic supplement to the 10T 1/2 cells.

1. Level II Amplification Assay

The amplification transformation assay involves replating the 10T 1/2 target cells at various times post-treatment and allowing them to re-establish confluence so that phenotypically transformed foci can develop on top of such confluent contact-inhibited cell monolayers. This procedure allows one to amplify the presence of transformed cells in the originally treated cell population such that the transformed phenotype can later be recognized, presumably as a function of (a) an extended expression time, and (b) the availability of greater numbers of

Table 5

Growth in Semi-solid Medium and Tumorigenicity of Transformed and Control Populations - Summary

POPULATION	EARLY PASSAGE (p3-5)		LATE PASSAGE (p16-18)	
	semi-solid medium growth	tumorigenicity	semi-solid medium growth	tumorigenicity
A	-	0/4	+++	0/5
B	+	0/4	+++	0/5
C	-	0/3	++	0/5
D	-	0/4	++	0/5
E	-	0/5	++	0/4
F	++	0/5	++	0/5
G	-	0/4	++	0/5
H		0/5	+++	0/5
I	-	0/3	++	0/4
J	-	0/4	+	0/4
L	-	2/5	+	0/3
N_1	-	0/5	++	0/4
N_2	±	0/5	+	1/5 (82)
M_1	-	0/4	++	0/5
M_2	±	0/5	+	0/5
S011/20 III	+	5/5	+++	4/5 (36-138)
S011/20 IT	+	9/10	+++	6/8 (18-33)
C3H-10T 1/2	-	0/6	-	0/7
serumless medium		0/8		0/8

Table 6

Comparison of Nude Mice and Irradiated Syngeneic Mice as Host Animals for Tumorigenicity Study of Transformed Populations

EXPERIMENT	POPULATION	SWISS NUDE MICE	IRRADIATED WEANLING (350r)
1	1229p2-3	1/3 (53)*	0/3
	1229p11-12	0/3	0/4
	1229p26-27	3/3 (67-81)	0/3
	S011/20 IT	3/3 (22)	9/10
	C3H-10T 1/2	0/2	0/6
	Serumless Medium	0/2	0/8
	POPULATION	**BALB/c NUDE MICE**	**IRRADIATED WEANLING (550r)**
2	B p16	0/3	0/3
	F p16	3/3 (35)	0/5
	H p16	0/3	0/3
	L p16	0/3	0/4
	M_2 p15	0/3	0/4
	S011/20 IT	2/2 (13)	3/3 (21-33)
	C3H-10T 1/2	0/3	0/2
	Serumless Medium	0/3	- -

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* The number in parenthesis is the number of days from the date of injection when tumor(s) is palpable.

cells at risk after treatment.

The compound which has served as a model for developing this approach was MNNG. The data on Table 7 show that in the standard (Level I) transformation assay little or no MNNG induced morphologically transformed Type III foci are observed. However, a marked enhancement of Type III focus formation is observed in the Level II Amplification Assay. These data suggest that the Level I MNNG treated cell population did indeed contain chemically transformed cells or cells in the process of being transformed, but that due to some as yet undefined limitations of the assay, these cells failed to express their transformed phenotypes. By simply amplifying their presence via the replating technique, the presence of transformed cells has been verified.

Use of this assay has enhanced the sensitivity of 10T 1/2 cells to the transforming effects of a variety of chemical carcinogens, some of which include dibenz(a,h)anthracene, aflatoxin B_1, lead acetate, 2-acetylaminofluorene (2-AAF), and diethylnitrosamine (DEN) (manuscript in preparation). The latter two agents also required an exogenous metabolic supplement to mediate induction of the Level II transformation response (see below).

To ensure that the 10T 1/2 cell response in the Level II transformation assay was, in fact, specific for carcinogenic chemicals, the amplification assay was also tested using a series of non-carcinogens. Phenanthrene and anthracene served as models (Table 8). The data show that either in the Level I standard assay or in the Level II amplification assay neither phenanthrene or anthracene were able to induce morphologically transformed foci in 10T 1/2 cells. On the other hand, the positive control, 3-methylcholanthrene, induced the formation of Type III foci in both the Level I and Level II procedures. These data thus indicate that the Amplification Assay is specific for chemical carcinognes and that the appearance of focus transformants in Level II is not a non-specific phenomenon related to indiscriminant chemical treatment.

2. S-9 mediated metabolic activation

The other major technological advancement currently being pursued is the use of an exogenous source of metabolic activation with which to supplement the 10T 1/2 cells in order to metabolically activate procarcinogenic chemicals to their bioreactive forms. Indeed, when the endogenous mixed function oxidase activity associated with 10T 1/2 cells was examined, that level was found to be relatively low (Table 9). In fact, relative to various other rodent cells (e.g. Syrian hamster embryo (SHE) and BALB 3T3 mouse embryo cells) the C3H 10T 1/2 cell line had very limited mixed function oxidase activity in terms of its ablity to convert organic soluble benzo(a)pyrene (BaP) to water soluble forms in a 24 hour period. Relative, for

Table 7

Detection of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) Induced Morphological Transformation of C3H 10T 1/2 Cl.8 Mouse Cells by the Standard Assay and the Amplification Assay in the Absence of Exogenous Metabolic Activation

Chemical	Dose (ug/ml)	RPE[a]	#CAR[b] ($\times 10^3$)	#T-III[c]	TF[d] ($\times 10^{-4}$)	DWF/TD[e]	
						Level I	Level II
Acetone, 0.25%	--	0.23	2.76	--	3.62	0/11 (<9.1)	0/36 (<2.8)
MNNG	1.0	--	--	0	--	0/11 (<9.1)	7/36 (19.4)
MNNG	0.5	0.16	1.92	1	5.21	1/12 (8.3)	9/36 (25.0)
3-MC	2.5	0.15	1.80	17	94.44	8/11 (72.7)	15/36 (41.7)

[a] RPE = relative plating efficiency, i.e. number of colonies per total number of cells seeded per condition in accompanying cytotoxicity assay.

[b] CAR = number of cells at risk after chemical treatment.

[c] T-III = number of morphologically transformed Type III foci per condition in Level I transformation assay.

[d] TF = transformation frequency, i.e. number of transformed Type III foci per number of cells at risk.

[e] DWF/TD = number of dishes with morphologically transformed Type III foci per total number of replicate dishes. (Numbers in parenthesis are percentages.)

Table 8

Detection of Phenanthrene and Anthracene Induced Morphological Transformation of C3H 10T 1/2 Cl.8 Cells by the Standard Assay and the Amplification Assay in the Absence of Exogenous Metabolic Activation[a]

Chemical	Dose ug/ml	% RPE[b]	#CAR[c] x10^3	III/TD[d]	TF[e] (x10^{-4})	DWF/TD[f] Level I	DWF/TD[f] Level II
Acetone (1 ul/ml)	-	19.0	2.85	0/15	<3.5	0/15	0/15
Phenanthrene	30	13.4	2.01	0/15	<5.0	0/15	0/15
Phenanthrene	10	15.6	2.34	0/15	<4.3	0/15	0/15
Phenanthrene	3	16.6	2.49	0/15	<4.0	0/15	0/15
Anthracene	30	16.2	2.43	0/15	<4.1	0/15	0/15
Anthracene	10	16.8	2.52	0/15	<4.0	0/15	0/15
Anthracene	3	14.9	2.24	0/15	<4.5	0/15	0/15
3-Methylcholanthrene	2.5	12.5	1.88	9/15	48.0	9/15	9/15

[a] Cells were seeded at 1 x 10^3 cells/dish, treated ± test chemical for 24 hr, washed and cultured with scheduled medium changes. Cells were fixed, stained and scored for morphologically transformed Type II and Type III foci after 4-6 weeks (Level I), or subcultured once at confluence approximately 2-4 weeks prior to scoring (Level II).

[b] RPE = relative plating efficiency, i.e. number of colonies per number of cells seeded per condition in accompanying cytotoxicity assay.

[c] $\bar{\text{CAR}}$ = number of cells at risk after chemical treatment.

[d] III/TD = number of Type III foci/total replicate dishes.

[e] TF = transformation frequency (Level I), i.e. number of Type III foci/#CAR.

[f] DWF/TD = number of dishes with Type III foci per total replicate dishes.

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Table 9

Benzo(a)pyrene (BaP) Metabolizing Activity of Various Mammalian Cells In Vitro

Cell Designation	mg Protein per dish	ug BaP Converted to H_2O-soluble Forms/ mg Protein/24 hr	Relative Metabolic Activity
SHE-12	0.726	8.800	1.000
CHO-K_1-BH_4	0.316	0.143	0.016
C3H 10T 1/2 C1.8	0.707	0.430	0.049
10T 1/2 S011-20Tumor	0.716	0.332	0.038
BALB 3T3 C1.A31-1	0.468	2.776	0.316
BALB 3T3 C1.A31-1C	0.492	2.927	0.333

example, to a Syrian hamster embryo primary culture, 10T 1/2 cells had about 20-fold less activity. Yet, despite this limited amount of activity, the 10T 1/2 cells still can metabolically activate the majority of polycyclic hydrocarbon carcinogens tested to forms which transform these cells (Table 3).

Nevertheless, repeated studies with other compounds, other than polycyclic hydrocarbons (for example, aromatic amines (e.g. 2-AAF), nitrosamines (e.g. DEN), chemotherapeutic agents (e.g. cyclophosphamide), etc.) have failed to yield any biological response when these 10T 1/2 cells were treated directly with the chemical in the absence of any metabolic supplement (data not shown). These results indicated that the 10T 1/2 cells apparently require metabolic supplementation in order to generate biologically active intermediates from such procarcinogens. To compensate for the limited endogenous metabolizing activity of the 10T 1/2 cells, efforts concentrated on the use of a 9000 x g postmitochondrial supernatant (S-9) preparation derived from an homogenate of Aroclor 1254 induced Fischer 344 (male) rat hepatic tissue.

Early studies with the chemotherapeutic agent, cyclophosphamide (Table 10), indicated that there was an S-9 concentration dependent, cyclophosphamide-induced, cytotoxic response by 10T 1/2 cells, with the maximal effect being observed at 20 ul S-9/ml (20 mg protein/ml). Time course studies also revealed that there was a time dependent cytotoxic response which was S-9 mediated and induced by cyclophosphamide, with a maximal effect being observed using a four hour treatment (Table 11). Similar time and S-9 concentration dependencies were observed with 2-aminoanthracene (Tables 12 and 13).

From such preliminary studies the metabolic activation transformation assay with 10T 1/2 cells using S-9 as the metabolic supplement was developed. Briefly, cells are seeded at a 1000 cells per (15 x 60)mm dish (transformation assay) and 250 cells per dish (concurrent cytotoxicity assay). Cells are treated for four hours at 37°C in the absence or presence of the metabolically functional S-9 preparation and an NADPH regenerating system. Following removal of the test chemical, cells are resupplied with fresh growth medium and maintained in culture with scheduled medium changes. Cells are fixed, stained and scored for (a) colony formation after 7-10 days (cytotoxicity assay) and (b) morphologically transformed Type II and Type III foci after 4-6 weeks (transformation assay).

Initial studies were performed with benzo(a)pyrene. Previous results have indicated that C3H 10T 1/2 cells can be transformed by BaP using $\geq$24 hour exposure periods. However, under the conditions of the activation assay using a four hour treatment, BaP alone in the absence of exogenous S-9 gave a rather limited focus transformation response (Table 14). In the presence of a metabolically functional S-9 preparation, however,

Table 10

Cytotoxic Effects of Cyclophosphamide (CPP) to C3H 10T 1/2 Cl.8 Cells in the Presence of Exogenous Metabolic Activity[a]

S-9[b] Concentration (ul/ml)	CPP Dose (ug/ml)	# Colonies/ 3 Replicates	% RPE[c]	% RCE[d]
20.0	0	202	26.9	100.0
20.0	5	25	3.3	12.4
6.7	5	104	13.9	51.5
2.2	5	185	24.7	91.6
0.7	5	207	27.6	102.5
0.2	5	200	26.7	99.0

[a] Cells were seeded at 250 cells/dish in EBME supplemented with 10% FBS and incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. Cells were treated for 4 hours at 37°C ± test chemical, ± S-9 and an NADPH-generating system, washed, cultured and scored for colony formation after 8-10 days.

[b] S-9 concentration (~20 mg protein/ml) was as indicated. The reaction mixture consisted of the cofactors NADH (0.5 mg/ml) NADP, NADPH and glucose-6-phosphate (0.75 mg/ml of each) in EBME + 2.5% FBS, pH 7.4.

[c] RPE = relative plating efficiency, i.e., number of colonies per total number of cells seeded per condition.

[d] RCE = relative colony-forming efficiency, i.e., number of colonies per condition relative to that of the control.

Table 11

Cytotoxic Effects of Cyclophosphamide (CPP) to C3H 10T 1/2 Cl.8 Cells in the Presence of Exogenous Metabolic Activity[a,b]

Exposure Time (min)	CPP Dose (ug/ml)	# Colonies/ 3 Replicates	% RPE[c]	% RCE[d]
240	0	202	26.9	100.0
0	5	178	23.7	88.1
15	5	198	26.4	98.0
30	5	178	23.7	88.1
60	5	124	16.5	61.4
240	5	7	0.9	3.5

[a] Cells were seeded at 250 cells/dish in EBME supplemented with 10% FBS and incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. Cells were treated for indicated times at 37°C ± test chemical, ± S-9 and an NADPH-generating system, washed, cultured and scored for colony formation after 8-10 days.

[b] S-9 concentration (~20 mg protein/ml) was 20 ul S-9/ml reaction mixture consisting of the cofactors NADH (0.5 mg/ml) NADP, NADPH and glucose-6-phosphate (0.75 mg/ml of each) in EBME + 2.5% FBS, pH 7.4.

[c] RPE = relative plating efficiency, i.e., number of colonies per total number of cells seeded per condition.

[d] RCE = relative colony-forming efficiency, i.e., number of colonies per condition relative to that of the control.

Table 12

Cytotoxic Effects of 2-Aminoanthracene (2-AA) to C3H 10T 1/2 Cl.8 Cells in the Presence of Exogenous Metabolic Activity[a]

S-9[b] Concentration (ul/ml)	2-AA Dose (ug/ml)	# Colonies/ 3 Replicates	% RPE[c]	% RCE[d]
20.0	0	202	26.9	100.0
20.0	5	0	0	0
6.7	5	6	0.8	3.0
2.2	5	83	11.1	41.1
0.7	5	166	22.1	82.2
0.2	5	180	24.0	89.1

[a] Cells were seeded at 250 cells/dish in EBME supplemented with 10% FBS and incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. Cells were treated for 4 hours at 37°C ± test chemical, ± S-9 and an NADPH-generating system, washed, cultured and scored for colony formation after 8-10 days.

[b] S-9 concentration (~20 mg protein/ml) was as indicated. The reaction mixture consisted of the cofactors NADH (0.5 mg/ml) NADP, NADPH and glucose-6-phosphate (0.75 mg/ml of each) in EBME + 2.5% FBS, pH 7.4..

[c] RPE = relative plating efficiency, i.e., number of colonies per total number of cells seeded per condition.

[d] RCE = relative colony-forming efficiency, i.e., number of colonies per condition relative to that of the control.

Table 13

Cytotoxic Effects of 2-Aminoanthracene (2-AA) to C3H 10T 1/2 Cl.8 Cells in the Presence of Exogenous Metabolic Activity[a,b]

Exposure Time (min)	2-AA Dose (ug/ml)	# Colonies/ 3 Replicates	% RPE[c]	% RCE[d]
240	0	202	26.9	100.0
0	5	177	23.6	87.6
15	5	186	24.8	92.1
30	5	165	22.0	81.7
60	5	157	20.9	77.7
240	5	6	0.8	3.0

[a] Cells were seeded at 250 cells/dish in EBME supplemented with 10% FBS and incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. Cells were treated for indicated times at 37°C ± test chemical, ± and an NADPH-generating system, washed, cultured and scored for colony formation after 8-10 days.

[b] S-9 concentration (~20 mg protein/ml) was 20 ul S-9/ml reaction mixture consisting of the cofactors NADH (0.5 mg/ml) NADP, NADPH and glucose-6-phosphate (0.75 mg/ml of each) in EBME + 2.5 FBS, pH 7.4.

[c] RPE = relative plating efficiency, i.e., number of colonies per total number of cells seeded per condition.

[d] RCE = relative colony-forming efficiency, i.e., number of colonies per condition relative to that of the control.

a marked (6-7fold) enhancement in the number of morphological transformants was observed (Table 14).

In a similar manner S-9 was also found to mediate 2-aminoanthracene(2-AA)-induced Type III focus transformation (Table 15). In the absence of a functional S-9, however, (elimination the NADPH regenerating system) no such transformants were observed.

These studies indicate that various known procarcinogenic chemicals can be metabolically activated to products which are capable of transforming C3H 10T 1/2 cells in culture. The universality of this technology is now being examined for diverse classes of chemical carcinogens requiring metabolic activation.

3. Amplification/Activation Transformation Assay

In some of the pilot studies conducted, there have been instances in which chemicals known to require metabolic activation failed to transform 10T 1/2 cells even in the presence of the S-9 supplement. In order to limit this possibility, studies have been initiated to combine the methodologies offered by the activation transformation assay with those of the amplification (Level II) assay. This technique thus far has proved efficacious for the detection of the transforming activity of the aromatic amine 2-AAF (Table 16). A metabolically functional S-9 was found to mediate the induction of Type III foci by a four hour treatment with 2-AAF (3.3 ug/ml). The focus response was observed only upon amplification in the Level II assay. No such activity was detected in the absence of a functional S-9 (cofactors deleted), even at Level II. Although the 10T 1/2 cell response was limited, the data exemplify situations in which the combined Amplification/Activation Transformation assay may be necessary to recognize the transformed phenotype.

Phenotypic transformation by diethylnitrosamine (DEN) was also found to require amplification after S-9 mediated metabolic activation to biologically reactive products (Table 17). The 10T 1/2 cells responded to a single dose of DEN (300 ug/ml), exhibiting Type III foci only in the Level II assay. In the absence of the S-9 preparation no such induction was observed.

E. CONCLUSION

The foregoing discussion has served to describe the C3H 10T 1/2 cell system and to evaluate its utility as a means of detecting chemical carcinogens. Having established the properties of the 10T 1/2 cells, including growth characteristics, in vivo tumorigenicity of chemically transformed cells, and endogenous hydrocarbon metabolizing capacity, it was our goal to assess the overall sensitivity and specificity of these cells to various carcinogenic chemicals. It was soon realized that

Table 14

Transforming Activity of Benzo(a)pyrene (BaP) to C3H 10T 1/2 Cl.8 Cells in the Presence of Exogenously Supplied Metabolic Activity[a]

Treatment	Chemical Dose (ug/ml)	RPE[b]	#CAR[c] ($x10^3$)	#T-II[d]	#T-III[e]	Transformation Frequency[f]		
						T-III/CAR ($x10^{-4}$)	T-III/td	dwT-III/td
S-9	--	0.14	1.68	0	0	<6.0	<0.08	<0.08
BaP	10.0	0.05	0.55	2	2	36.4	0.18	0.18
BaP + S-9	10.0	0.10	1.20	6	13	108.3	1.08	0.67
BaP + S-9	5.0	0.11	1.21	1	11	90.9	1.00	0.64
BaP + S-9	2.5	0.15	1.50	0	1	6.7	0.10	0.10
BaP + S-9	1.0	0.18	1.98	1	0	<5.1	<0.08	<0.08

[a] Treatment time was 4 hr.

[b] RPE = relative plating efficiency, i.e. number of colonies per total number of cells seeded per condition in accompanying cytotoxicity assay.

[c] #CAR = number of cells at risk per treatment condition.

[d] #T-II = number of Type II transformed foci.

[e] #T-III = number of Type III transformed foci.

[f] Expressed as Type III foci/cells at risk (T-III/CAR), Type III foci/total replicate dishes (T-III/td), and dishes with Type III foci/total replicate dishes (dwT-III/td).

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Table 15

Transforming Effects of 2-Aminoanthracene (2-AA) to C3H 10T 1/2 Cl.8 Cells in the Presence of Exogenous Metabolic Activation[a]

Treatment	2-AA Dose (ug/ml)	#T-III/td[b]	(TF)	dwT-III/td[c]	(TF)
2-AA + S-9 + cofactors	3.00	3/15	(0.2)	3/15	(0.2)
2-AA + S-9 + cofactors	1.50	2/30	(0.07)	2/30	(0.07)
2-AA + S-9 + cofactors	0.75	0/30	(<0.03)	0/30	(<0.03)
2-AA + S-9 + cofactors	0.38	3/30	(0.10)	1/30	(0.03)
2-AA + S-9 + cofactors	0.19	0/30	(<0.03)	0/30	(<0.03)
S-9 + cofactors	0	0/30	(<0.03)	0/30	(<0.03)
2-AA + S-9 - cofactors	1.50	0/15	(<0.07)	0/15	(<0.07)
2-AA + S-9 - cofactors	0.38	0/15	(<0.07)	0/15	(<0.07)

[a] Exposure time was 4 hr.; S-9 concentration was 20 ul S-9/ml (20 mg protein/ml).

[b] #T-III/td = number of Type III foci/total replicate dishes; numbers in parentheses are transformation frequencies (TF).

[c] dwT-III/td = dishes with Type III foci/total replicate dishes; numbers in parentheses are transformation frequencies (TF).

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Table 16

Detection of S-9 Mediated 2-Acetyl Aminofluorene (2-AAF) Induced Morphological Transformation of C3H 10T 1/2 Cl.8 Cells by the Standard (Level I) Assay and the Amplification (Level II) Assay[a]

Treatment[b]	2-AAF Dose (ug/ml)	RPE[c] (%)	#CAR[d] x10^3	TRANSFORMATION FREQUENCY			
				T-III/TD[e]		DWF/TD[f]	
				Level I	Level II	Level I	Level II
S-9 + cofactors	0	12.1	1.815	0/15	0/15	0/15	0/15
2-AAF + S-9 + cofactors	10.0	3.2	0.480	0/15	0/15	0/15	0/15
2-AAF + S-9 + cofactors	3.3	6.4	0.960	0/15	2/15	0/15	2/15
2-AAF + S-9 + cofactors	1.0	4.1	0.615	0/15	0/15	0/15	0/15
2-AAF + S-9 + cofactors	0.3	7.3	1.095	0/15	0/15	0/15	0/15
2-AAF + S-9 - cofactors	10.0	10.1	1.515	0/15	0/15	0/15	0/15
2-AAF + S-9 - cofactors	3.3	10.8	1.620	0/15	0/15	0/15	0/15

---

[a] Cells were seeded at 1 x 10^3 cells/dish, treated for 4 hr ± test chemical, ± S-9 and an NADPH-generating system, washed and re-supplied with fresh medium every 3-4 days. Cells were fixed, stained, and scored for morphologically transformed Type II and Type III foci after 4-6 weeks (Level I), or subcultured once at confluence approximately 2-4 weeks prior to scoring (Level II).

[b] S-9 concentration (~20 mg protein/ml) was 20 ul S-9/ml reaction mixture consisting of NADH (0.5 mg/ml), NADP, NADPH and glucose-6-phosphate (0.75 mg/ml of each), in EBME + 2.5% FBS, pH 7.4.

[c] RPE = relative plating efficiency, i.e., number of colonies per number of cells seeded per condition in accompanying cytotoxicity assay.

[d] CAR = number of cells at risk after chemical treatment.

[e] T-III/TD = number of Type III foci per total replicate dishes.

[f] DWF/TD = number of dishes with Type III foci per total replicate dishes.

Table 17

Detection of S-9 Mediated Diethylnitrosamine (DEN) Induced Morphological Transformation of C3H 10T 1/2 Cl.8 Cells by the Standard (Level I) Assay and the Amplification (Level II) Assay[a]

				TRANSFORMATION FREQUENCY			
				T-III/TD[e]		DWF/TD[f]	
Treatment[b]	DEN Dose (ug/ml)	RPE[c] (%)	#CAR[d] x10^3	Level I	Level II	Level I	Level II
S-9 + cofactors	0	17.4	2.610	0/15	0/15	0/15	0/15
DEN + S-9 + cofactors	1000	17.5	2.625	0/15	0/15	0/15	0/15
DEN - S-9 + cofactors	1000	18.9	2.835	0/15	0/15	0/15	0/15
DEN - S-9 + cofactors	300	19.6	2.940	0/15	0/15	0/15	0/15
DEN + S-9 + cofactors	1000	10.9	1.635	0/15	0/15	0/15	0/15
DEN + S-9 + cofactors	300	13.4	2.010	0/15	4/15	0/15	3/15
DEN + S-9 + cofactors	100	16.5	2.475	0/15	0/15	0/15	0/15

---

[a] Cells were seeded at 1 x 10^3 cells/dish, treated for 4 hr ± test chemical, ± S-9 and an NADPH-generating system, washed and re-supplied with fresh medium every 3-4 days. Cells were fixed, stained, and scored for morphologically transformed Type II and Type III foci after 4-6 weeks (Level I), or subcultured once at confluence approximately 2-4 weeks prior to scoring (Level II).

[b] S-9 concentration (-20 mg protein/ml) was 20 ul S-9/ml reaction mixture consisting of NADH (0.5 mg/ml), NADP, NADPH and glucose-6-phosphate (0.75 mg/ml of each), in EBME + 2.5% FBS, pH 7.4.

[c] RPE = relative plating efficiency, i.e., number of colonies per number of cells seeded per condition in accompanying cytotoxicity assay.

[d] CAR = number of cells at risk after chemical treatment.

[e] T-III/TD = number of Type III foci per total replicate dishes.

[f] DWF/TD = number of dishes with Type III foci per total replicate dishes.

specific assay modifications would be required to render these cells a generally applicable screening system. Through the collaborative efforts of the laboratories at both Arthur D. Little, Inc. and Microbiological Associates, it was determined that suitable modificatons of the C3H 10T 1/2 cell transformation assay included (1) cell synchronization, (2) variations in target cell density, (3) target cell replating, and (4) use of exogenous sources of metabolizing activity (e.g. primary hepatocytes or S-9). Each of these appraoches has proven feasible for certain classes of chemical carcinogens. It will now be necessary to established suitable technical procedures for use as generalized screens for unidentified potential carcinogens. Such decisions must await the assessment of the sensitivity and utility of each of these procedures in identifying diverse classes of genotoxic agents.

F. REFERENCES

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2. Reznikoff, C.A., Bertram, J.S., Brankow, D.W. and Heidelberger, C. Quantitative and qualitative studies of chemical transformation of cloned C3H mouse embryo cells sensitive to postconfluence inhibition of cell division. Cancer Res. 33: 3239-3249, 1973.

LEGEND

Figure 1. Protocol for Standard Neoplastic Transformation Assay with C3H 10T 1/2 Cells

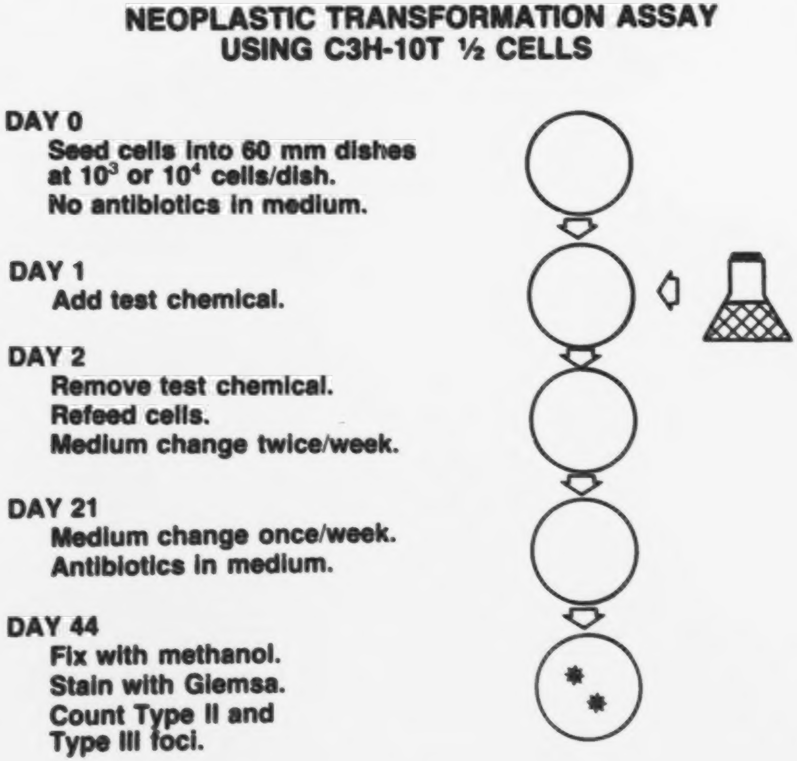
Figure 2. Type III Transformed Focus. Giemsa-stained, X.

Figure 3. Comparison of Transformation Response to 3-Methylcholanthrene in Collaborating Laboratories

Figure 4. Cell Cycle Dependency of Cytotoxicity and Transformation of C3H 10T 1/2 Cells Treated with MNNG (1 ug/ml, 10^5 Plating Density)

Figure 5. Comparison of MCA Metabolism by Primary Rat Hepatocytes and C3H 10T 1/2 Cells

Figure 6. Cyclophosphamide Cytotoxicity on C3H 10T 1/2 Cells in the Presence and Absence of Primary Rat Hepatocytes - A. C3H 10T 1/2 - Untreated; B. C3H 10T 1/2 + Hepatocytes - Untreated; C. C3H 10T 1/2 - Treated (cyclophosphamide 100 ug/ml); D. C3H 10T 1/2 + Hepatocytes - Treated (cyclophosphamide 100 ug/ml).



**NEOPLASTIC TRANSFORMATION ASSAY
USING C3H-10T ½ CELLS**

DAY 0
Seed cells into 60 mm dishes at 10^3 or 10^4 cells/dish.
No antibiotics in medium.

DAY 1
Add test chemical.

DAY 2
Remove test chemical.
Refeed cells.
Medium change twice/week.

DAY 21
Medium change once/week.
Antibiotics in medium.

DAY 44
Fix with methanol.
Stain with Giemsa.
Count Type II and Type III foci.

FIGURE 1. Protocol for Standard Neoplastic Transformation Assay with C3H-10T 1/2 Cells

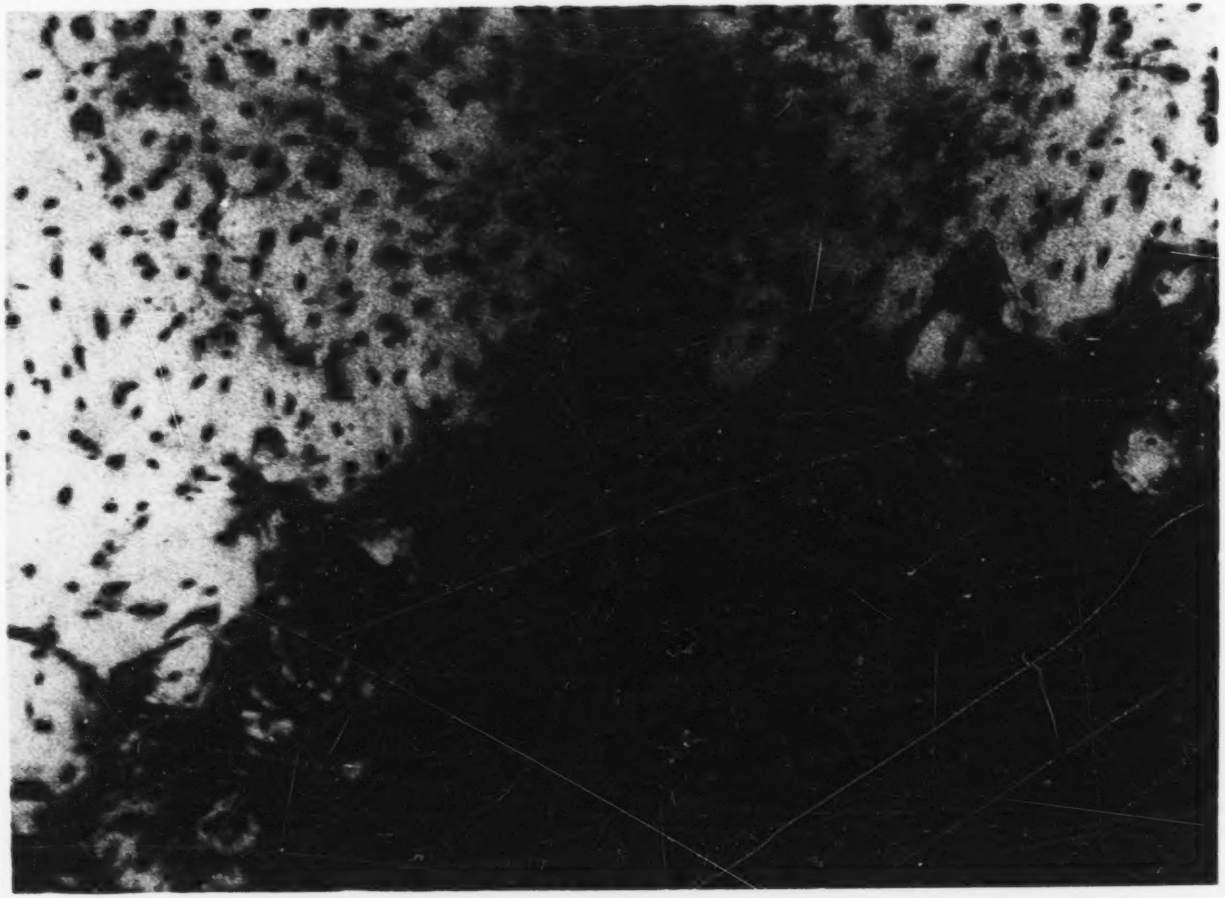


FIGURE 2. Type III Transformed Focus. Giemsa-stained, X.

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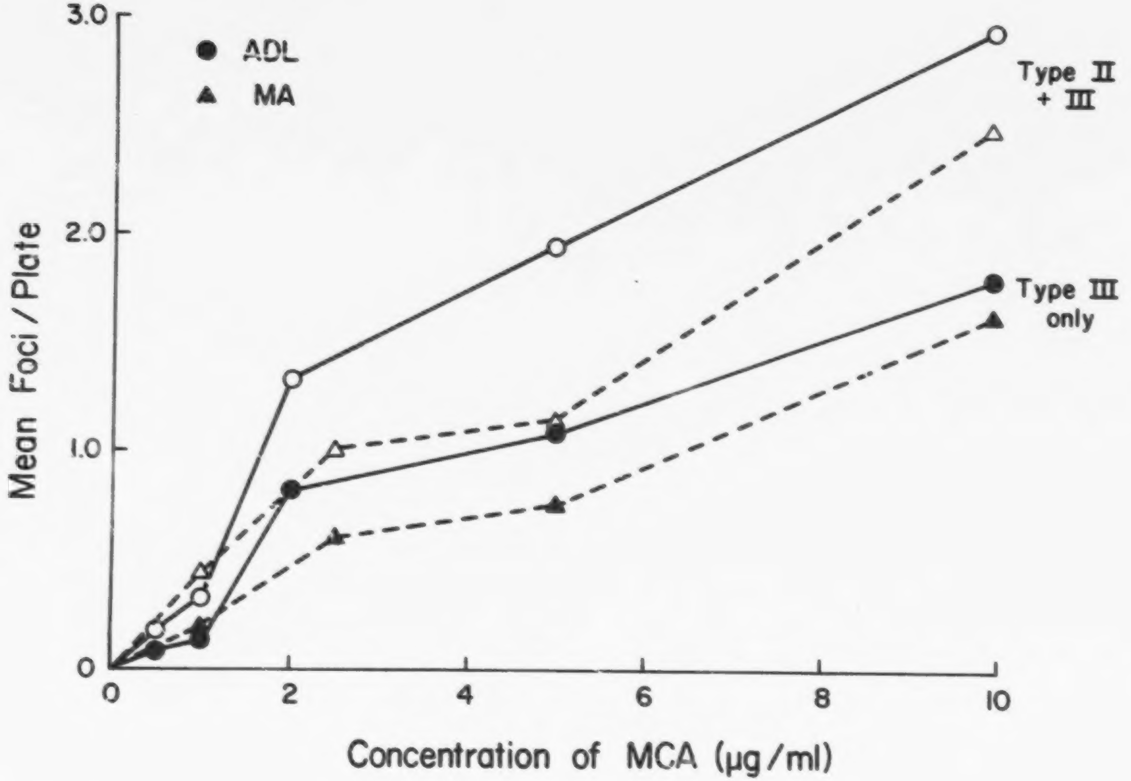
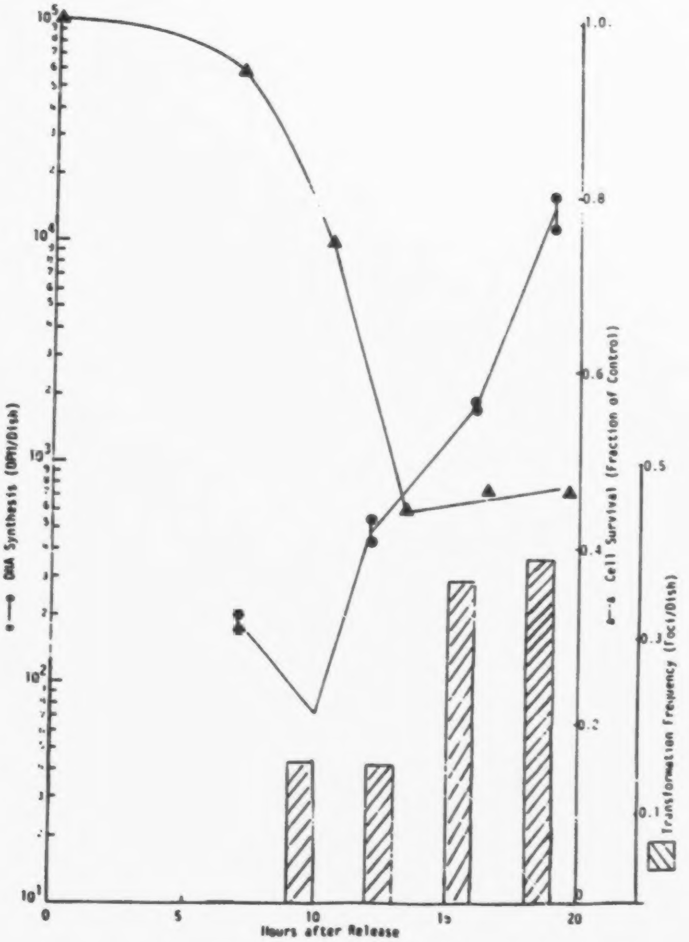


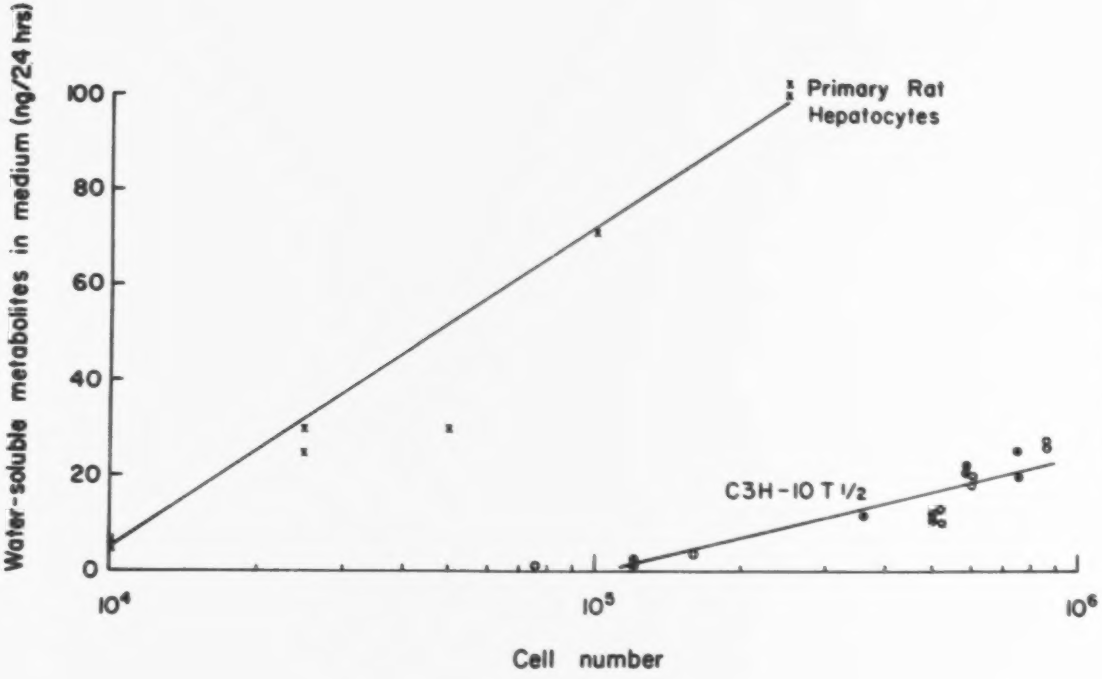
FIGURE 3. Comparison of Transformation Response to 3-Methylcholanthrene Collaborating Laboratories

FIGURE 4

Cell Cycle Dependency of Cytotoxicity and Transfomration of C3H-10T 1/2 Cells Treated with MNNG (1 μg/ml - 10^5 Plating Density)



Cell Cycle Dependency of Cytotoxicity and Transformation of C3H-10T 1/2
Cells Treated with MNNG (1 μg/ml - 10^5 Plating Density)



COMPARISON OF MCA METABOLISM BY PRIMARY RAT HEPATOCYTES AND C3H-10T 1/2 CELLS

FIGURE 5. Comparison of MCA Metabolism by Primary Rat Hepatocytes and C3H-10T 1/2 Cells

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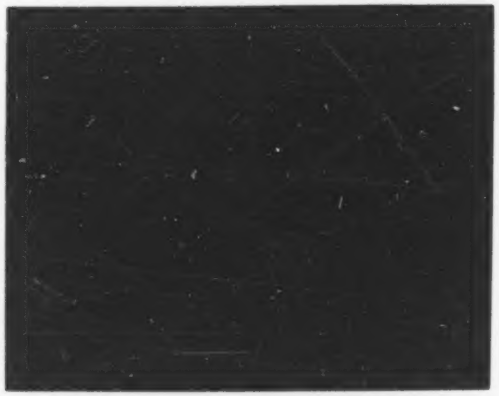
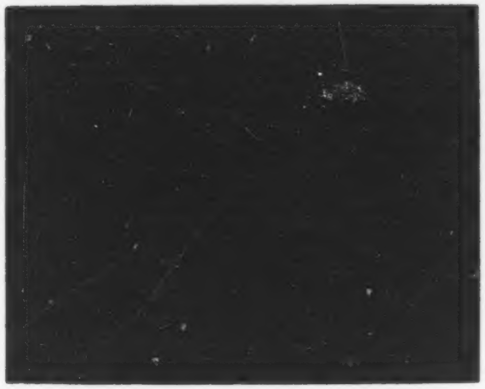
A. No Hepatocytes



C. No Hepatocytes



B. With Hepatocytes

D. With Hepatocytes

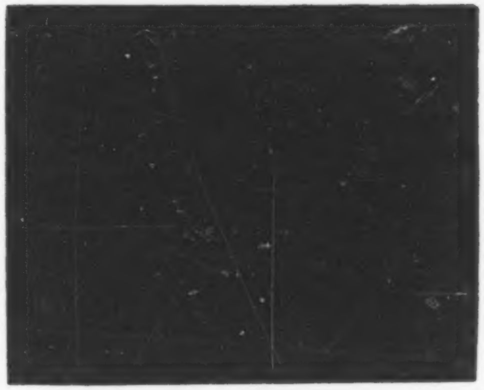


FIGURE 6. Cyclophosphamide Cytotoxicity on C3H-10T 1/2 Cells in the Presence and Absence of Primary Rat Hepatocytes - A. C3H-10T 1/2 - Untreated; B. C3H-10T 1/2 + Hepatocytes - Untreated; C. C3H-10T 1/2 - Treated (cyclophosphamide 100 μg/ml); D. C3H-10T 1/2 + Hepatocytes - Treated (cyclophosphamide 100 μg/ml)

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DR. MENTER: I would like to ask a question concerning the types of inducers you are planning on using. I notice that you concerned yourself with an aroclor type of induction. Are you looking for other species or inducers, as well as other animals?

DR. SCHECHTMAN: Okay, the initial studies concentrated on the rat hepaticus using aroclor 1254. We are also getting involved in -- or are involved in the hamster S-9 preparation which may or may not need induction, depending upon the chemical being screened, and there are certain agents which we have come across using other assay systems where we have found, as a matter of fact, that we have to induce with the test chemical itself. So aroclor 1254 is not the be all and end all as far as inducers go. There are, obviously, others that will have to be examined, those which elevate hydrase activity, things of that nature.

DR. MENTER: Well, I was going to suggest your priming the animal with the test agent itself. Also I would like to mention some work that we have recently completed where we have been able to demonstate MMNG transformation in 10T1/2 mouse cells simply by treating the cultures five days after seeding, without having to synchronize the cells. By doing this we have been able to demonstrate at least one focus per dish and about 60 to 70 percent of dishes with foci. I believe that this is probably due to the density dependence, that it is having more cells at risk.

DR. SCHECHTMAN: That is true because MNNG in the 10T1/2 cell is quite cytotoxic. I imagine Dr. Sivak can address that.

DR. SIVAK: That is absolutely correct. The one slide I didn't show, I skipped over, exactly showed that point, that as you go to higher cell densities with these direct acting agents, you do begin to see activity. I am not sure what the reason is. I suspect it may be that you are cell cycling that part of the population that is not constrained by the rest and you may be having a larger cell population going through the S-phase at the time, whereas, the 103 cells, you have a very low number of cells that are going through at an early time.

DR. WEISBURGER: A very quick question, do you know whether these cells have stayed more or less stable over the ten years since they have been introduced, you know, the 10T1/2 cells?

DR. SIVAK: They are very stable, yes. They need to be husbanded, they need to be extremely well taken care of, like any biological system; great care must be taken as to passages and how they are used. But if they are well taken care of, they will last for a long time.

PROCEEDINGS OF THE
SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Hairless Mice for Carcinogenesis Studies

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INTRODUCTION

Skin cancer incidence is relatively low among heavily pigmented people; even among caucasians under equivalent insolation there is a great deal of variability in the incidence of solar damage, including carcinogenesis. The Celts have long been recognized as a population particularly susceptible to UVR-induced skin cancer. Whether this difference is a function of, or merely correlated with this ethnic group's cutaneous characteristics, cannot yet be resolved. Heritable differences in skin tumor susceptibility may not be solely a function of pigmentation, cornified tissue thickness and other obvious anatomic features; the nature of the heritable difference is of great practical interest.

There is a limited amount of comparative species information available on photocarcinogenesis. The larger mammals appear to have skin tumor development time roughly proportional to life expectancy. Smaller laboratory rodents (rats, hamsters, mice), particularly those without hair, have shorter lifespan and shorter skin tumor development time, and are thus preferred for the saving they represent in time and money.

Two strains of albino hairless mice show distinctly different responses to the carcinogenic stimulus of broad spectrum UVA plus 8-methoxypsoralen (8-MOP) (Grube et al 1977). In contrast, these strains had similar dose-response relationships for such acute symptoms as erythema and epidermal cell thymine dimer crosslink formation.

We have found that groups of hairless mice, having similar anatomic features but differing in genetic background, can be significantly different in their susceptibility to tumors induced by simulated sunlight. These groups were exposed simultaneously and their acute reactions were very similar.

This report describes how we have measured susceptibility to UVR-induced skin cancer in mice, and refers to our current efforts to identify possible sources of their dissimilar UVR-cancer susceptibility, such as subtle differences in skin structure, in epidermal cell kenetics, in vascular response, or in immunologic capability.

MATERIALS AND METHODS

Design: All of the studies reported here used UVR irradiation with the same spectral distribution (from a xenon arc solar simulator filtered with 1 mm of Schott WG 320 doped glass) but the design included studies with two types of dose delivery. In both cases the animals received daily (5 days per week) whole body exposures described here as "high-yield studies" and "dose-response studies".

1. The high-yield studies involved a daily dose high enough to produce a perceptible response (mild edema, followed by flaking and hyperplasia) but not high enough to produce ulceration and wound healing phenomena. All animals received the same daily dose, and the variable was the genetic background of the animals. The rationale of this type of study was to derive directly comparable information on several stocks and strains of animals as quickly as possible and this turned out to be a period of approximately six months.

2. Among the dose-response types of study, each stock and strain of animals was exposed to four different daily doses which covered a range of 18-50% of the dose described above. Each experiment involved animals of one genetic type, with the dose per day as the variable. The aims of this type of study included the determination of the range of responses for each of the stocks and strains, and an analysis of whether the dose-response curve implied any different mechanisms which might be associated with interactive processes (e.g. promotion, repair, etc.). With this type of study, cross comparisions among stocks and strains are possible, but some allowance must be made for unintentional variables among studies that are not run simultaneously (seasonal changes, tumor counters with differences in visual perception, etc.).

Animals: The mice used in these studies were obtained from the animal services division of the Skin and Cancer Hospital (Temple University School of Medicine). They are described in the addendum.

Irradiation Conditions: The custom-built light source used in these studies has been described elsewhere (Forbes, 1978). Briefly, a 6kw water-cooled Xenon arc lamp, model Rm-60, was mounted vertically with the mid-arc located 115 cm above the floor. Free-moving mice were individually housed in cage compartments which were designed for horizontal irradiation: mobile racks suspended the cages with their fronts (exposed surfaces) located at the perimeter of a circle, two meters from the central light source. The total front-surface area of occupied cages on each cage rack was 120 x 100 cm. For this study the light source was surrounded, at a distance of 20 cm, by a stationary octagonal metal frame designed to hold a 15 x 15 cm glass filter in each side, thus creating an 8-sector filtered source; the corresponding segments of the perimeter of the circle provided the location for specific treatment groups (one treatment group per cage rack). A Schott glass filter (1mm of #WG-320) was used to simulate mid-latitude sunshine.

The exposure of each group was monitored by simultaneous exposure of a Robertson-Berger sunburn ultraviolet (R-B) meter. In addition, spectral emission curves were obtained at approximately monthly intervals (and whenever a burner or filter was replaced) for each emission sector using a comparison spectroradiometer accessory of a Cary Model 14 spectrophotometer.

The R-B meter incorporates a detector whose spectral sensitivity is greatest in the "sunburn" portion of the solar UVR spectrum (<320 nm). The meter has been used to estimate the biological effectiveness of sunlight exposure, with 400 counts on the accumulator mechanism being defined as a "sunburn unit" (SU); this value was chosen because under specified conditions (clear summer midday sun, sea level, latitude 20-27°S) it corresponded to the exposure duration (12 minutes) required to produce minimal erythema in "normal" skin; thus the estimated solar flux under those conditions was 5 SU per hour.

The R-B meter was chosen to serve three functions. The first was to guide in the choice of UVR doses, attempting to avoid ineffectiveness on one extreme and tissue distruction on the other extreme. The second was to monitor daily exposures with each spectral source (this required multiple matched detectors). The third was to provide one recognized weighting function (instrumental) against which to compare the response being studied (photocarcinogenesis). For absolute energy units, the Cary spectroradiometer described above was considered the primary reference.

Procedures for Photocarcinogenesis Studies: Animals were observed twice daily and were individually weighed and examined weekly. Cutaneous changes were recorded graphically on outline drawings and in narrative form. Localized thickenings of the epidermis could be recognized as developing tumors when they reached approximately 0.5 mm in diameter; upon first detection they were assigned individual numbers, recorded on the outline drawings. Subsequent examinations included measurement of maximum diameter by calipers. Notations were made on the records when each of three diameter thresholds (1,2 or 4mm) was reached; subsequently the absolute value of the maximum diameter was recorded.

Data were transfered to computer memory by manual (keyboard) entry, using an interactive program which incorporated both mechanical and visual checks of data validity. All such transfers were made by a single individual whose functions include verification and validation of data gathering operations on a weekly basis. The numeric data storage format for tumor records included individual numbered entries for each tumor on each animal, indicating the week at which each size category was attained, the week on which the tumor merged with another (specified) tumor and, for tumors which disappeared, the week of loss and notation of whether loss was clearly traumatic (injury, ulceration etc.) or apparently "spontaneously". In addition, for each animal the week of last observation was recorded, with a coded entry where appropriate to indicate the reason for loss removal, the condition and disposition of the carcass and whether or not histologic specimens were obtained.

Data were sumarized in two ways. Cumulative tumor incidence was defined as $(S_a + D_a)/(S_a + S_u + D_a)$, where S_a and S_u were the numbers of survivors either affected or unaffected with respect to the defined tumor category and D_a was the number of animals which has previously died after producing one or more qualifying tumors. These data were calculated on a weekly basis and fitted to a sigmoid curve (the logistic transform) against the logarithm of experiment week as an index of exposure. Data were fitted by the method of minimum logit chi-squares, and the resulting equation was used to estimate the median latent period (T_{50}).

Tumor multiplicity or yield was defined as the average number of qualifying tumors present on surviving animals; the numerator included tumors which had been lost (very few) or disappeared due to merger (fairly frequent at high multiplicity). The square root of calculated weekly yield was plotted against experiment time (linear), and a best-fit linear relationship for non-zero values was calculated by least squares (using number of survivors as the weighting function). The resultant line was used to estimate the time (T_1) at which surviving animals exhibited and average of one qualifying tumor. Except at very low doses the numeric values of T_1 and T_{50} were similar.

RESULTS AND DISCUSSION

High-yield studies: Even allowing for natural variability, some rather striking differences are apparent in the tumor response among the various stocks and strains of mice. The animal types listed in Table 1 showed the responses in Figure 1. Differences appear to be associated with several characteristics such as the mutant in question, the presence of skin pigment, as well as one or more unidentified factors.

In addition to hairless (hr/hr) we studied animals affected by three mutations which bring about the condition of lacking hair on the surface of the skin.

These are the genes named Asebia (as/as), Cryptothrix (crh/crh), and Rhino. Rhino is an allele of hairless, and we worked with the hairless/rhino combination which produces an intermediate phenotypic expression (please refer to description in addendum). The hairless/rhino animals are not shown in the figure, but their response was very similar to that of asebia and cryptothrix. The average epidermal thickness in cryptothrix and hairless/rhino mice is significantly greater than that of comparably aged hairless mice. The asebic mice have a relatively thin epidermis, but they have a residue of hair stubble at the surface of the skin. Whether the hair stubble accounts for the resistance to UV-induced tumors is not known.

In general, albino mice tend to be more susceptible to UV-induced cancer than those mice which have some melanocytic activity. Although the body skin of mice with pigmented hair, eyes, tails and ears contains very little melanocytic activity, the mice do have delayed tumor appearance. This is particularly evident in comparisons between Skh:hr types 1 and 2 mice, which have a common genetic background other than the pigment expression. Hairless mice from the pigmented C3H background also appear to express some resistance to UV carcinogenesis. Whether this is directly a function of the pigment itself, and if so whether the mechanism is by way of absorption or scattering of the UV has not been tested.

Mice from the inbred albino HRA/Skh line have a significantly shorter latent period for tumor production than mice from the HRS/J inbred albino line. Whether this represents a greater "susceptibility" on the part of HRA mice, or greater "resistance" on the part of the HRS mice is not known, and is more than a matter of semantics. Identifying either the source of the resistance or susceptibility could enhance our understanding of the carcinogenic process.

As inbred lines go, the HRA/Skh hairless mice represent an acceptably prolific and robust line, with an extremely low background of "spontaneous" skin tumors.

Dose-response studies: As described in the methods section, four response curves were derived for each genetic group and these four lines (one for each daily dose level) tended to form a set of approximate parallel lines. This was consistent with our previous findings on Skh:HR mice that were irradiated with fluorescent sunlamps (Forbes et al 1981). One method of summarizing this kind of information is to determine the time to 50% incidence for each of the curves, and plot the reciprocal of each of these values against weeks in the experiment. One sample of this kind of analysis is shown in Figure 2. In this case, the most sensitive of the hairless lines (HRA/Skh) is compared with the least sensitive (HRS/J) and also with the relatively insensitive hairless/Rhino intermidiate (hr hr^{rh}). From this type of analysis, it would appear that the differential sensitivities of these animal lines are represented by proportional offsets, and that the slopes of the responses are parallel. This has some interesting implication for the kinetics of tumor formation among the several stocks and strains of animals.

Immunology: The stocks and strains listed above were evaluated for ability to respond to two mitogens (concanavalin A and lipopolysaccharide) and to two antigens (sheep erythrocytes and pneumococcal polysaccharide, type 3). The numbers of T and B lymphocytes in the thymus, spleen and pooled lymph nodes of individual animals were determined using immunofluorescent techniques; the values for each group of animals were found to be comparable. The antibody-forming ability was evaluated by determining the number of plaque-forming cells in the spleen after immunization with either antigen. All strains of mice responded to both antigens as well as to the mitogens (Smith et al 1981). The same tests were run on animals from these stocks and strains after they had been irradiated daily for 15 weeks with the dose as described under "high-yield studies" above. Again, all of the tested animals responded to the antigens and to the mitogens, with no clear relationship to the corresponding susceptibility to UV-induced tumors. We conclude therefore that the animals tested have the type of immunologic response one would associate with normal mice, and that if there is an immunologic association to tumor susceptiblilty, that the effect is too subtle to be detected by these screening evaluations.

Another approach to the question of immunologic effect is by using mice which are immunologically incompetent, but reconstituted with immune anlage from different donor strains. Irradiation and evaluation of such reconstituted animals has not yet proceeded to the point where we can offer conclusions on their apparent tumor sensitivities.

Acute Responses: Selected strains are being evaluated in terms of vascular reactions (edema, ear thickening) and in terms of epidermal responses (sunburn cell formation, epidermal cell kinetics). The studies are still underway, and the data have not therefore been evaluted.

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TABLE I *Characteristics of test animals*

Stock or strain	Designation	Relevant genetics[a] Hair growth	Color	Source	Response curve # (Fig 3)
Stock	Skh:HR (type 1)	hr/hr	c/c	S&C Temple Univ.	1
	Skh:HR (type 2)	hr/hr	+/c, +/b, +/a	S&C Temple Univ.	9
Strain (F18)	HRA/Skh	hr/hr	c/c	S&C Temple Univ.	2
Stock	Skh:CRH	+/crh	+/c	S&C Temple Univ.	3
Strain (N8, F3)	C3H/HeN-hr	+/hr		NIH	6
Strain (F80)	HR/De/Hflcr	+/hr	p/p, b/b	Inst. Cancer Res.	5
Strain (F54)	HRS/J	+/hr	c/c	Jackson Lab.	7
Stock	Argonne hairless	+/hr	c/c	Argonne Nat. Lab.	8
Strain (F81+37)	BALB/cSkh-ab	+/ab	c/c, b/b	Univ. of California, Berkeley to S&C Temple Univ.	4

[a] Genotype symbols, c-albino, ab-asebia, b-brown, a-non agouti, crh-crytpothrix, hr-hairless, p-pink eyed.

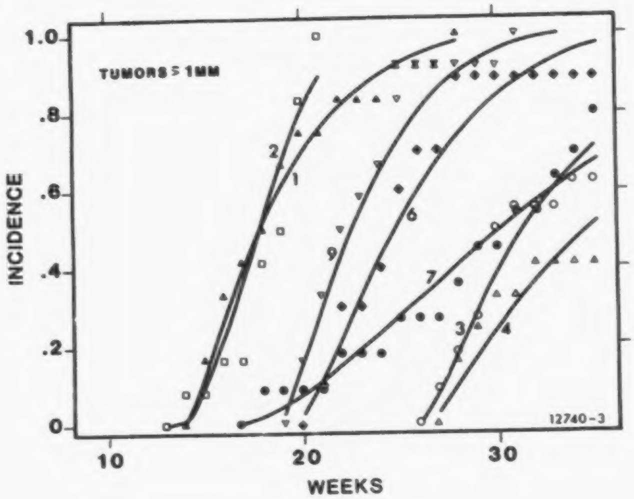

Fig. 1

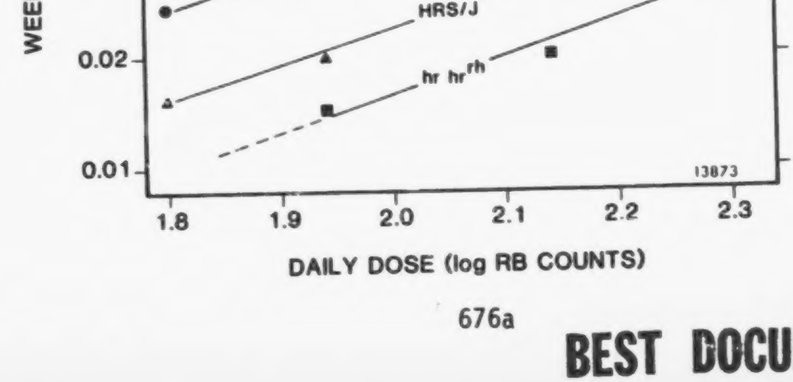

Fig. 2

ADDENDUM

Stock: Skh:HR (types 1 and 2)

Genetics: The stock is homozygous for the mutation hairless (hr; chromosome 14). The stock also carries the mutations albino (c), brown (b) and non-agouti (a), which segregate out in a variety of phenotypes. For convenience, all albino (c/c) offspring are referred to as "type 1" or Skh:HR-1, and all pigmented offspring are called "type 2" or Skh:HR-2. Among the latter, mice can be recognized as agouti or non-agouti (based on their juvenile hair coat), and either black or brown (based on juvenile hair coat, or on the ear and tail color of mature mice). All pigmented mice have only one pigmented parent, and are therefore heterozygous at the c locus.

Origin and History: Non-descript hairless mice were obtained in 1968 from Sandra Biological Supply, a now defunct company. These were outcrossed once with the CBA/Man inbred strain. Subsequent breeders were selected to maintain a stock with desired characteristics: number and size of litters born and successfully nursed by homozygous mutant dams; robust offspring; a regulatable proportion of pigmented vs. albino offspring. The stock is maintained by standardized "random cross" technique.

Mating System: Homozygous hairless females are mated with homozygous hairless males. Matings are by paired animals; infrequently matings are two females with one male. All offspring are of the hairless phenotype. The proportion of type -1 breeders is kept large in order to meet the greater demand for albino test animals.

Housing and Disease Problems: The stock is maintained in Jackson Lab style double stainless steel pens in a convential closed colony system. The stock has no known health problems.

Breeding status and delivery of mutant offspring: Skh:HR-1 and -2 are routinely used at Temple. Adequate albinos and pigmented mice are presently available for study.

Breeding Characteristics: The Skh is a vigorous and prolific stock of hairless mutant mice.

TABLE 1

Litter Size Among Parities of Skh:HR-1

Parity*	No of Litters	Total born ± S. E.	Total weaned Female ± S. E.	Total weaned Male ± S. E.	% Wean	(sex ratio) % Female wean
1	50	9.2 ± 0.3	3.6 ± 0.3	4.2 ± 0.3	83.7	46.1
2	50	10.2 ± 0.4	4.4 ± 0.4	3.9 ± 0.3	80.5	52.9
3	50	10.6 ± 0.4	4.3 ± 0.3	4.3 ± 0.4	81.0	49.8
4	50	9.5 ± 0.5	3.7 ± 0.3	4.0 ± 0.3	81.6	48.2
5	49	8.8 ± 0.5	3.6 ± 0.3	3.5 ± 0.3	81.0	50.6
6	44	7.8 ± 0.6	3.6 ± 0.3	3.2 ± 0.3	86.9	53.2
7	26	7.2 ± 0.4	2.6 ± 0.2	3.8 ± 0.3	89.3	40.1

Table 1 (continued)

Litter Size Among Parities of Skh:HR-2

Parity	No of Litters	Total born ± S. E.	Total weaned Female ± S. E.	Total weaned Male ± S. E.	% Wean	(sex ratio) % Female Wean
1	40	9.4 ± 0.3	3.7 ± 0.3	4.5 ± 0.9	88.3	45.0
2	40	9.9 ± 0.5	4.8 ± 0.4	4.3 ± 0.4	91.4	52.9
3	39	9.6 ± 0.5	4.8 ± 0.3	4.1 ± 0.3	92.0	54.0
4	35	10.3 ± 0.5	4.9 ± 0.4	4.2 ± 0.3	88.3	53.5
5	24	9.9 ± 0.5	4.6 ± 0.4	4.2 ± 0.3	88.2	52.4

*Parity number is the nth litter born to a breeding pair

Strain: HRA/Skh (F23, 1981)

Genetics: This inbred strain is homozygous for the hairless (hr) mutant gene.

Origin and History: The orgin is the same as for the Skh:HR stock. Albino mice from the outbred stock were crossed brother with sister. Selection was made for litter size and nursing ability of the homozygous dam. The coefficient of inbreeding is 0.99. At F13, these animals permanently retained exchange skin grafts.

Mating System: One homozygous hairless (hr/hr) female is mated with one homozygous hairless (hr/hr) male. All resulting offspring are of the hairless mutant genotype.

Housing and Disease Problems: Same as for the Skh stocks.

Breeding status and delivery of mutant offspring: The pedigree foundation colony has been expanded and an expansion colony has been established. Groups of 200 mice within a week of age are now being delivered.

Breeding Characteristics: See Table 2. Average litter size at birth is about six which is somewhat smaller than for the outbred Skh:HR stock (Table 1). All experimental animals will be related by a common great grandmother.

Table 2

Litter Size Among Parities of HRA/Skh Hairless Mice

Parity	No of Litters	Total born ± S. E.	Total weaned Female ± S. E.	Total weaned Male ± S. E.	% Wean	(sex ratio) % Female wean
1	39	5.6 ± 0.3	2.2 ± 0.2	2.8 ± 0.4	88.6	44.6
2	24	6.6 ± 0.6	2.4 ± 0.4	2.6 ± 0.4	74.8	47.9
3	18	7.4 ± 0.6	2.7 ± 0.4	2.3 ± 0.5	79.9	44.9

Strain: HRS/J

Genetics: Inbred F54 (?). The strain carries the following alleles: hr, b, c, d. Albinism is common to all members of the strain.

Origin and History: The hairless stock originally came from Crew to Carnochan, to Heston, to Chase, to E. L Green, to Les, to M. C. Green, to Jax, to Skin and Cancer 1978. Animals received by Skin and Cancer were not pedigreed but came from about the F54 generation. In addition to the hairless allele, this strain shows a high frequency of leukemia in the mutant offspring; 45% at 8 to 10 months; 72% at 18 months. In +/hr, there is a 1% incidence of leukemia at 10 months and 20% at 18 months.

Mating System: Heterozygous hairless females are mated with homozygous males. Pedigrees are being maintained on brother x sister foundation matings.

Housing and Disease Problems: The strain has remained in isolation since arrival at Skin and Cancer. There appears to be no health problem within the strain. The mice are maintained in transparent polycarbonate plastic cages in a conventional housing system.

Breeding Status and Delivery of Mutant Offspring: The original animals were received March 29, 1978 and consisted of eight haired females and seven homozygous hairless males. The colony has been expanded; Groups of 60 mice within one week of age are delivered for photobiology studies.

Breeding characteristics: See Table 3. Even with the limited available data, the reproductive capacity of the HRS/J strain is poor. Moreover the litter sizes shown in Table 3 include both mutant and normal offspring. The hairless mutant mice represent about one half of the data. The colony exhibits cycles of low productivity. Homozygous hairless females are fertile but mammary gland development is poor and young are not raised. Six mutant females were bred and all six had at least one litter. Young were killed and eaten at birth or shortly after birth.

Table 3

Litter Size Among Parities of HRS/J

Parity	No of Litters	Total born ± S. E.	Total weaned Female ± S. E.	Total weaned Male ± S. E.	% Wean	(sex ratio) % Female wean
1	16	3.2 ± 0.5	1.9 ± 0.4	0.9 ± 0.3	66.7	67.4
2	10	5.6 ± 0.6	2.9 ± 0.3	2.2 ± 0.5	93.9	54.5

Strain: HRS/Anl

Genetics: Partially inbred: generation unknown. Except for hr, and c, nothing is known about the color alleles. Albinism is common to all members of the "strain".

Origin and History: Received at S&C June 1978 in response to our request for HRS/Anl breeders. The HRS/Anl originated with the HRS/J strain which was sent to the Argonne National Laboratories from the Jackson Laboratory in about 1963. The animals initially were mated brother with sister but for years have been random mated; anecdotal evidence suggests some outcrossing. Apparently little exists in the way of pedigree records. The animals received by the Skin and Cancer Hospital were not pedigreed. These animals differ somewhat from the HRS strain (see Breeding Characteristics), and thus the strain designation is no longer consistant with standard nomenclature.

Mating System: Heterozygous hairless females are mated with homozygous hairless males. Pedigrees are being maintained on brother x sister foundation matings. Some matings have been made, and are being maintained, using homozygous hairless females mated with homozygous hairless males.

Housing and Disease Problem: The "strain" has remained in isolation since arrival at the Skin and Cancer Hospital. There appear to be no health problems. The mice are maintained in transparent polycarbonate plastic cages in a convential housing system.

Breeding Status and Delivery of Mutant Offspring: The original animals were received June 1, 1978 and consisted of ten haired females and ten homozygous hairless males. The colony has been expanded. Groups of 60 animals within one week of age have been used.

Breeding Characteristics: See Table 4. Females of the HRS/Anl are excellent breeders, better than HRA/Skh strain and almost equal to the outbred Skh:HR stocks. The HRS/J and the HRS/Anl differ significantly in breeding performance (Table 3 and Table 4). Since the HRS/J was highly inbred when sent to Anl, the modified reproductive capacity was most likely a function of outcrossing. Moreover, mating of homozygous hairless females with homozygous hairless males indicate that the homozygous females are fertile, have large litters and are very capable of raising their young. From 5 matings, each female had a litter for an average of 5.6. All 28 young born to these females were weaned.

During the last year we received breeding pairs of HRS/Anl mice that had been maintained at the Oak Ridge National Laboratory. These are being compared with those described above.

Table 4

Litter Size Among Parities of HRS/Anl

Parity	No of Litters	Total born ± S. E.	Total weaned Female ± S. E.	Total weaned Male ± S. E.	% Wean	(sex ratio) % Female wean
1	21	7.3 ± 0.5	3.5 ± 0.4	2.6 ± 0.4	84.3	57.4
2	12	7.7 ± 0.8	2.7 ± 0.5	4.2 ± 0.7	90.2	39.8

Strain: HR/De/HfIcr

Genetics: Inbred F 80. Carries br, p.

Origin and History: An outbred stock from Crew to Carnochan, to Deringer who inbred the strain, to Hayflacker, to Institute of Cancer Research 1948, to Skin and Cancer July 21, 1978. The animals that were received by Skin and Cancer were not pedigreed but they came from the F80 generation. The mutant mice develop papillomas and hemiangioendotheliomas. There is a low incidence of mammary tumors.

Mating System: Heterozygous hairless females are mated with homozygous males. Pedigrees are being maintained on brother x sister matings.

Housing and Disease Problems: The mice are in isolation. There appears to be no health problems. The mice are maintained in polycarbonate plastic cages in a conventional housing system.

Breeding Status and Delivery of Mutant Offspring: The animals were received July 21, 1978 and consisted of fifteen heterozygous hairless females and fifteen homoyzgous hairless males. Several dozen mutant hairless mice have been delivered for pilot studies.

Breeding Characteristics: In our facilities the breeding capacity of this strain is low. The offspring are smaller and less robust than those of the other strains, and too few have been produced for large-scale studies.

Strain: C3H/HeN-hr (N8)

Genetics: hr

Origin and History: The hairless allele has been back crossed onto the C3H/HeN background by the NIH Laboratory Animal Genetics Center. Except for the hr allele, these mice are of the typical black agouti wild type. The animals received by the Skin and Cancer Hospital on June 27, 1978 were not pedigreed but they came from the eighth backcross generation. The coefficient of inbreeding is 0.996 and will be considered inbred onto the C3H Strain.

Mating System: Until now, the matings consisted of an outcross of hairless male to C3H female and a subsequent sib intercross to produce another generation of hairless males which were again outcrossed to C3H females. The strain will be pedigreed and maintained by mating homozygous hairless males with their heterzygous hairless sisters.

Housing and Disease Problems: The strain has remained in isolation since arriving at Skin and Cancer. Neonatal mice have infantile diarrhea. From the symptoms, the strain appears to be infected with epidemic diarrhea of infant mice (EDIM). However, there is a possibility that the strain is infected with lethal intestinal virus of infant mice (LIVIM) or a combination of both viruses. Either viral type could have been transmitted by other mice recently received for this project. Most young survive, they continue to nurse and show typical "pot belly" associated with EDIM. Young adults appear healthy.

We will continue to breed these animals but there is no possibility of their being moved into the inner core of the breeding facility. These animals will have to be cleaned up by caesarean derivation if they are to be of any long-term value. The animals are maintained in polycarbonate plastic cages with filter top bonnets in a convential system.

Breeding Status and Delivery of Mutant Offspring: The animals were received June 27, 1978 and consisted of 9 heterozygous hairless females and four homozygous hairless males. The colony has been expanded and groups of 60 within a week of age have been delivered.

Breeding Characteristics: Litter size is moderate, probably similar to the C3H inbred strain.

Stock: Cryptothrix (Skh:crh)

Genetics: (c,a crh)

Orgin and History: The mutation occured in the Charles River Breeding Laboratories COB-1 outbred albino stock. Initally the mutation was thought to be hairless (hr) but proved to be a new mutation at a separate locus. There is only one report in the literature concerning this mutation (J. Invest. Dermat. 56:170, 1971). The initial mutant animals were received at Skin and Cancer Hospital in 1967 from Charles River.

Mating System: The original stock of mice was maintained for a number of generations with a limited number of brother x sister matings. Inbreeding was not successful. Homozygous mutant females were infertile and in time, homozygous mutant males became infertile. The mutation was outcrossed with the C57BL/10 inbred strain which improved fertility. The mutation was not used for a number of years and was maintained in a very small outbred colony. By 1977, the fertility of the stock was very poor. The stock has again been outcrossed to the C57BL/10 strain. The mutation is maintained on an outbred genetic background.

Housing and Disease Problems: The major part of the stock is maintained in the inner core of the breeding colony. There is no known disease. The mice are maintained in Jackson Lab style double stainless steel pens in a convential system.

Breeding Status and Delivery of Mutant Offspring: The colony has been expanded and significant number of offspring put into studies.

Breeding Characteristics: This mutation has had cyclic phases of fecundity during the last ten years. Even when the stock was on a highly outbred background homozygous mutant female would not raise their young. However, homozygous mutant males are good breeders when on an outbred genetic background. The mutation probably could be established on an inbred background, if an adequate starting pool of breeders were available.

Strain: BALB/cSkh-ab (F81 +37)

Genetics: c, b, ab

Origin and History: The asebia (ab) mutation was a spontaneous mutation occuring in the BALB/cCrglGa inbred strain. The mutation occured in the F74 and was sublined thereafter. The new subline was designated BALB/cGa-ab. The strain went from the University of California, Berkley to Stanford University School of Medicine and was received by the Skin and Cancer Hospital in 1967. The strain was at the F81 generation of brother to sister matings. There have been a limited number of publications concerned with asebia mutant mice (Science 148:1471, 1965; J. Invest. Dermat. 52:115, 1969; J. Invest. Dermat. 52:119, 1969).

Mating System: Pedigreed brother with sister matings. The ab is maintained in forced heterozygosity on the BALB/c inbred strain. Matings are generally made with the mutant female mated to the heterozygous male or the heterozygous female mated to the homozygous male.

Housing and Disease Problems: The strain is maintained in the inner core of the breeding facility. There are no known diseases. The animals are housed in Jackson style double stainless steel pens in a convential system.

Breeding Status and Delivery of Mutant Offspring: The foundation colony had consisted of only six to eight breeding females. The colony has been expanded and significant numbers of offspring incorporated into studies.

Breeding Characteristics: Mutant mice of either sex are fertile and females have no problem rearing their young.

Stock: hairless-rhino compound (hr/hr^{rh})

Genetics: Individuals of this genotype are produced by crossing homozygous hairless (hr/hr) females of the Skh:HR-1 with homozygous rhino males (hr^{rh}/hr^{rh}) of the Skh:rhino-2 stock (see following). All offspring are of the hr/hr^{rh} compound genotype. The phenotypic characteristics of the integument such as epidermal thickness, number of hair cellial cysts and skin wrinkling are intermediate between that of hairless and rhino mutant mice.

Breeding status and delivery of mutant offspring: Hairless/rhino compound mutant mice can be easily and quickly produced. Readily available Skh:HR females are mated to rhino male mice.

Stock: Skh:rhino-2

Genetics: Rhino (hr^{rh}), a multiple allele of hairless (hr), is located on chromosome 14. All members of the Skh:rhino-2 stock are black (BB), and either agouti or non-agouti.

Origin and History: The rhino mutant stock was obtained from the Jackson Laboratory by Mann at Brown University in 1958, brought to Roswell Park Memorial Institute in 1961 and to the Skin and Cancer Hospital in 1965. The orginal allele was backcrossed for a number of generations onto the C57BL/10Ck inbred strain. This line was designated Skh:rhino-1 but was discontinued in 1972. The reproductive capacity of the females became so poor that an outcross was necessary to save the mutant gene. Mutant rhino males of the Skh:rhino-1 were crossed with CBA/Man females. The resulting offspring were maintained on a outbred background. A selection program for this stock, Skh:rhino-2, was established to produce females who produce large litters and nurse their young.

Mating system: Heterozygous females are mated with homozygous males. One half of the offspring are of the mutant genotype and one half are heterozygous for the rhino allele. Attempts to select homozygous females who nurse their young have not been successful.

Housing and Disease Problems: The stock is maintained in Jackson style double stainless steel pens in a conventional closed colony. There are no known health problems.

Breeding Status: The stock had been maintained as a small nuclear breeding colony which could be expanded as needed.

Breeding characteristics: See Table 5. The selection program was successful in producing a vigorous stock of mice; homozygous females do not nurse their young. In a total of 45 litters, there were 162 rhino and 155 normals produced which is a good fit for an expected 1:1 ratio. In all parities, there is an excess of females.

TABLE 5

Litter Size Among Parities of Skh:rhino-2

Parity*	No of Litters	Total born ± S. E.	Total weaned Female ± S. E.	Total weaned Male ± S. E.	% Wean	(sex ratio) % Female wean
1	28	8.5 ± 0.4	3.9 ± 0.4	2.9 ± 0.3	79.4	57.7
2	17	9.3 ± 0.6	4.4 ± 0.4	3.1 ± 0.5	77.1	58.6
3-4-5	26	9.1 ± 0.5	4.0 ± 0.4	3.4 ± 0.4	81.4	53.9

Strain: C57BL/6-hr^{rh} (N8)

Genetics: hr^{rh}, a/a. All members of this strain are black, non-agouti

Origin and history: The origin of the hr^{rh} allele is the same as for the Skh:rhino-2. Males for Skh:rhino-2 were crossed to females of rh C57BL/6 Man inbred strain. Sibs from the female offspring were intercrossed and male rhino offspring were again crossed with C57BL/6 females. The coefficient of inbreeding is 0.99+. Since there have been eight backcrosses and eight sib crosses, the mutant gene will be considered as being inbred onto the C57BL/6 background and will be referred to as the C57BL/6-hr^{rh} strain.

Mating system: Heterozygous female are mated to heterozygous males. Mutant males are mated to C57BL/6 females.

Housing and Disease Problems: The strain is maintained in Jackson style double stainless steel pens, automatic watering system in a conventional colony.

Breeding Status and Characteristics: The strain is maintained in a small foundation colony. Although there is only a limited amount of breeding data, cursory evaluation indicates that females are fair breeders. The colony could be expanded to produce any quantity of needed young.

Stock: HRH/Skh (F11)

Genetics: Hr^{rh}, All members of this strain are black agouti

Origin and History: The origin of the hr^{rh} allele is the same as for Skh:rhino-2. Mating pairs from the outbred Skh:rhino-2 were bred brother to sister. The inbreeding has continued for eleven generations. The coefficient of inbreeding is 0.91. The stock will be referred to as the HRH/Skh, indicating the intention to develop an inbred strain.

Mating System: Homozygous males are mated to heterozygous female sibs. One half the offspring are of the rhino genotype. The haired members are heterozygous for rhino.

Housing and Disease Problems: The stock is maintained in Jackson style double stainless steel pens in a conventional closed colony. There are no known health problems.

Breeding Status and Characteristics: Since the stock is maintained as a foundation colony, breeding data are limited; cursory evaluation indicates that females are fair breeders.

SPEAKER: Do you think the differences in line 7 and 1 will carry over to anything beside UV with X-rays or any chemical carcinogens? Do you think it is systemic or immunologic?

DR. FORBES: We believe that there are differences in chemical carcinogenesis between the lines and we are at the moment looking to see if we can distinguish between initiation and promotion both with photocarcinogenesis and chemical carcinogenesis.

PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Effects of Carcinogens, Mutagens, and Teratogens on Non-Human Species (Aquatic Animals)

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Third Annual Report

NCI/EPA Collaborative Program

Fiscal Year 1981 (May, 1980 - October, 1981)

Project 3

EFFECTS OF CARCINOGENS, MUTAGENS, AND TERATOGENS ON NON-TARGET SPECIES - (AQUATIC ANIMALS)

by

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EFFECTS OF CARCINOGENS, MUTAGENS, AND TERATOGENS ON NON-HUMAN SPECIES-AQUATIC ANIMALS

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Aquatic systems and organisms are under both laboratory and field study in order to develop indicator, screening, and modeling capabilities for detection and evaluation of risks of carcinogens, mutagens, and teratogens. Studies include both Gulf Breeze laboratory projects and complementary, extramural projects. In the third year of the program, several advances were made in the development of laboratory and field carcinogen assays, utilizing fishes such as the sheepshead minnow (liver lesions via benzidine and aflatoxin exposures), rainbow trout (liver tumors via benzo(a)pyrene exposures), and freshwater catfish (papillomatous-like lesions via chlorinated effluent exposures). Emphasis is being placed on the development and utilization of critical life stage exposures (e.g., embryo exposures) in order to expedite carcinogen tests and minimize time required for tumorogenic responses. Results of long-term exposures of fish to the herbicide trifluralin show that induced boney growths (in vertebral columns) are accompanied by enlargement and histopathologic changes of the pituitary. A novel approach has shown that tiger salamanders may be good biochemical and histologic indicators of the presence of certain carcinogens (polycyclic aromatic hydrocarbons - PAH's). Skin and liver tissues of the salamanders revealed induced enzyme activity (MFO system) following exposure to the PAH, perylene. Considerable field monitoring work on mollusks and carcinogenic PAH's along the coast of Oregon has revealed a positive correlation between prevalence of cellular proliferation disorders in shellfish and higher concentrations of certain PAH's in natural water. Emphasis in biochemistry for the last year has been directed mostly toward the elucidation of the metabolism of the mixed-function oxidases in marine organisms. From continuing work on the mullet (*Mugil cephalus*), we have found that benzo(a)pyrene (B(a)P) is converted mainly to 3-hydroxybenzo(a)pyrene, 9-hydroxybenzo(a)pyrene and 4,5-, 7,8-, 9,10-dihydrodiols of BaP, in *in vitro* systems containing liver microsomes from Aroclor 1254-treated mullet. Other metabolites such as diones of B(a)P and probably tri- and tetraols have also been produced. We have also developed a procedure for the epoxide hydrolase enzyme system in order to determine the role of this enzyme on the metabolism of the polyaromatic hydrocarbons. Our studies show that UDP-glucuronosyl transferase and sulfotransferase were significantly increased in livers of rats given phenobarbital, phenolphthalein, phenanthrene, BaP or 3-MC orally for 10 to 14 days. The injection of PB or 3-MC into rats, sea catfish or mullet on long-term schedule resulted in increase in liver size, and an increase in UDP-glucuronosyl transferase in livers of all three animal species. We have observed that the liver microsomal fraction of the killifish, *Fundulus grandis*, has a high *in vitro* activity when injected with a single dose of 3-MC, converting B(a)P to 1-OH, 3-OH, 5-OH, 6-OH, 7-OH, (t) 9,10-diol, (t) 7,8-diol, 1,6-, 3,6- and 6,12-diones, and 4,5-oxide. There may be an optimal concentration of NADPH, and this concentration may have a significant effect on the products of the in vitro reaction, quantitatively as well as qualitatively. These studies continue to reveal that fish have metabolic pathways similar to mammals for disposition of certain carcinogens. A brief study was conducted to determine possible interactions between the 9-OH BaP and salmon sperm DNA. The interaction between salmon DNA and 9-OH BaP was shown by relative fluorescence studies. The binding was evidenced by an eight-fold reduction in relative fluorescence yield. We are developing methods to determine the possible binding of proximal carcinogenic metabolites to DNA's of selected aquatic animals.

III. Introduction

A major problem faced by the National Cancer Institute and the Environmental Protection Agency is that of aiding in determining the fate and effects of carcinogenic pollutants in the larger environment. One way to approach the problem of understanding risks of general subject exposure is to study wildlife populations that are widespread, but which live in environments where exposure to ambient pollutants is certain. The use of wildlife populations as surrogates for human populations may be considered to be a novel expansion or logical extension of the use of laboratory animals and animal models of human diseases as alternatives to use of human subjects. The sharing of biologic characteristics by phylogenetically diverse species makes certain comparative approaches possible.

Problems arise, however, when such factors as selection of sensitive, indicative species, geographically adequate populations, and representative segments of the air, land, water biosphere are considered. In this regard, Office of Research and Development laboratories, such as Gulf Breeze Environmental Research Laboratory, have exemplary, pilot research programs that are investigating the use of aquatic animal species as indicators of carcinogens in the environment. The Gulf Breeze pilot research program has been under way since August, 1978 and is supported jointly by the Office of Research and Development (EPA) and the National Cancer Institute through an interagency agreement. The Gulf Breeze studies are based on the premise that the aquatic portion of the biosphere (water, biota, and sediment) is the ultimate "sink" for the runoff, fallout, and discharge of most toxic pollutants. In addition, animals living in the relatively efficient solvent, water, are more intimately exposed (total exposure through body surfaces, gills, alimentary tracts) than are species living in terrestrial or air environments. Aquatic species are also less likely to escape a dissolved or carried pollutant.

At Gulf Breeze, researchers are studying species of fish and shellfish along the Northern Gulf of Mexico (Florida, Alabama, Mississippi) in order to determine which are good indicators of the role of carcinogenic agents in the environment. Selected species of fish are exposed in long-term tests in the laboratory to determine their specific tissue, cellular, and biochemical responses to known chemical carcinogens that may occur in the environment. In addition, a significant number of cooperative agreements with principal investigators from around the nation support an extramural complemental and supplemental effort in the identification of aquatic species and systems that may serve as early warning mechanisms.

IV. Summarized Objectives

Objective 1:

Determine fate and effects of carcinogens, mutagens, and teratogens in aquatic species (individuals; populations).

Objective 2:

Determine role of aquatic species and systems in actual or potential exposure of man to carcinogens, mutagens, or teratogens (metabolism, accumulators).

Objective 3:

Develop aquatic species as bioindicator, sentinel, and model systems for use in study of risks of carcinogens, mutagens, and teratogens in the general environment (sensitive species and systems).

V. Methodological Approaches

We have underway both in-house and complementary extramural research projects. The overall project is divided into two major disciplinary approaches: 1) Pathobiology and 2) Biochemistry. Therefore, methods outlined below and results in the next section will be reported under these two complementary, disciplinary headings. Detailed results of each cooperative agreement in progress during FY 81 will be included at the end of this report. The disciplinary area of each cooperative agreement will be identified by project officer (Couch - pathobiology; Schoor - biochemistry).

1. Pathobiological Methods

Fish carcinogen assay system, toxicology, and histopathology of induced lesions.

At Gulf Breeze, both long-term (12-24 months) and short-term (1-6 months) carcinogen exposure systems were designed and tested. The long-term system provided exposure of all cycle stages of fish development (embryo to adult) during a complete life cycle experiment. Continuous injection of known concentration via a syringe-injection apparatus has permitted exposure of *Cyprinodon variegatus* (Sheepshead minnow) to the herbicide Trifluralin for 21 months (Conc. = 1-5 μg/l). The short-term systems provided exposure of all life-cycle stages of fish (embryo to adult) during complete life stage development. The short-term systems provided exposure of select embryologic states (Fig.1). Short-term, static exposures for minutes to hours of embryos of *C. variegatus* to the carcinogen aflatoxin were completed and the fish are being grown to adults in clean water for study of possible tumor development.

In extramural efforts, other fish carcinogen assay systems are being developed by Hendricks in Oregon (rainbow trout), and by Martin in Mississippi (*C. variegatus* in static and recirculating systems). Grizzle in Alabama is utilizing both laboratory and field approaches to study the causation and development of tumor-like lesions in catfish by taking advantage of populations of yellow bullheads in sewage treatment ponds. Rose and Anderson are developing an assay system, utilizing both tumor induction and enzyme induction as end points, with the tiger salamander (*Ambystoma tigrinum*)as the subject.

The preceding carcinogen tests cover the range from early life stage (zygote and embryo) exposure to complete life-cycle exposures (embryos through adulthood) and involve both field studies (*in situ*) and laboratory tests on a wide variety of compounds with overt or suspect carcinogenic activities.

A long-term monitoring and field study of neoplastic diseases in mollusks in Oregon coastal waters is continuing. This study, based on careful sampling of shellfish, sediments, and water, is investigating possible links in pollutant activity in estuaries and tumor-like diseases in bivalve mollusks (see report by Mix in addenda). The results of a two-year study of disease incidence and pollutant prevalence in three Gulf coast estuaries are being analyzed. Diagnosis of lesions in fishes and invertebrates are being completed, and chemical analytical data are being summarized by Lawler and Lassater at the University of New Orleans.

2. Biochemical Methods

Induction Studies: Aquatic species such as mullet, killifish, flounder, sea catfish and others have been and will continue to be exposed to inducers of microsomal mixed-function oxygenase (MFO) activity either by intraperitoneal injection or direct exposure in seawater. Since periods up to one year might be necessary to induce MFO activity by water exposure, the direct injection of inducer is used at the start of the investigations in order to optimize other parameters such as metabolite and conjugation reactions. The long-term, low exposure in seawater will follow. [Schoor, Melius (CR809493), Strength (CR809673)]. Metabolite Identification: Metabolites from the MFO reactions will be identified using high pressure liquid chromatography coupled with fluorescence detection and confirmed by stopped-flow fluorescence scanning. Metabolite standards for benzo(a)pyrene [(BaP)] have been obtained from the Illinois Institute of Technology through the courtesy of NCI. All the phenolic compounds have been chromatographed and their excitation and emission spectra have been obtained in appropriate solvents. All spectra will be stored on discs for later data manipulation. Conjugation and Excretion Studies: Rats are being used to make a series of conjugation products *in vivo* by injection of ^{14}C-labelled BaP. They will include glucuronides, glutathiones, and sulfates. Their occurrence in fish will then be ascertained by comparison to the standards produced in the rat. This will be helpful in determining the final disposition of a carcinogen-like BaP within the animal and in what forms the parent compound is finally passed back into the seawater.

VI. MAJOR FINDINGS AND PROGRESS

As noted earlier under Methodological Approaches, results and findings of studies to date are reported under the two disciplinary areas of Pathobiology and Biochemistry with extramural cooperative agreement reports at the end of this report.

1. Pathobiology

Long-term (21 months) exposures of sheepshead minnows to the herbicide Trifluralin (a suspect carcinogen) were completed, lesions diagnosed and assessed and a final report written on the efficacy of the long-term, flow-through carcinogen assay system (Couch, Courtney, Foss, 1980). We now consider this system state-of-art for use at Gulf Breeze for long-term exposures of fish and select invertebrates to suspect carcinogens. Though no neoplasms were induced by Trifluralin in the sheepshead minnows, a variety of other lesions including vertebral column dysplasia, and pituitary hypertrophy were consistently produced at low concentrations during the continuous life-cycle exposure. None of the control fish had these lesions.

During FY 81, we began the first critical life-stage pulse exposures of the sheepshead minnow to aflatoxin to determine its responsiveness to a known, potent carcinogen. Ninety-six hour old embryos (liver tissue present) were exposed for one hour to 200 µg aflatoxin B_1/l. One hundred and twenty hour old hatchlings were also exposed for one hour to 200 µg aflatoxin B_1/1. These fish suffered insignificant mortality from the pulse exposure. Following exposure, fish were placed in clean sea water and are now being grown to adulthood and studied for tumor development with an emphasis on hepatic lesions. Histologic examination of tissues from one month old fish (exposed as embryos) revealed an increase in hepatocyte mitotic activity (3x - 4x normal, based on controls). It is too early to determine overt preneoplastic lesions in the fish. Samples will be taken at monthly intervals until fish are one year old. The response of the sheepshead minnow to aflatoxin will be compared to the known responses of rainbow trout and other vertebrates to similar exposures.

Studies using recirculating exposure systems have shown that severe liver lesions, probably adenofibrotic or neoplastic, are characteristic responses of the sheepshead minnow to 1 ppm benzidine, a known carcinogen. (Martin, 1981 - see attached cooperative agreement report CR 86212). The severity of these lesions is remarkable and extensive within the liver and have characteristics of hemangioendotheliomas, cholangiomas, or bile duct adenomas.

Rainbow trout exposed to dietary benzo(a)pyrene (100 ppm) have developed hepatocellular carcinoma and preneoplastic liver lesions consisting of basophilic nodules. This work, done at Oregon State University (Hendricks, 1981 - see attached cooperative agreement report CR 809344) is the first example of tumor induction by benzo(a)pyrene in a fish species.

Grizzle at Auburn University found that black bullhead catfish in an oxidation pond of a sewage treatment plant in Alabama had 70% prevalence of oral papillomas. A concentrate of water from this pond was mutagenic to bacteria in the Ames test. Biochemical studies indicated liver dysfunction and mixed function oxidase induction in fish with tumors (see attached report CR 807844010).

Anderson and Rose are studying perylene (a PAH) metabolism and tumor induction _in vitro_ and _in vivo_ in the tiger salamander (_Ambystoma_), an inhabitant of contaminated effluent ponds in Texas. A system was developed to quantify perylene (P) metabolism _in vitro_ by _Ambystoma_ microsomal preparations. Perylene metabolism had not previously been studied in any animal. Both liver and skin samples can metabolize P in untreated animals. Perylene hydroxylase activity in both sites can be significantly induced by prior treatment with polychlorinated biphenyls or 3-methylcholanthrene. Perylene, like BaP, is a promutagen which requires metabolic activation by microsomal enzymes to give a positive reaction in the Ames _Salmonella_ mutagenesis assay. The ability of _Ambystoma_ aryl hydrocarbon hydroxylase (AHH) to generate mutagenic P derivatives seems to be very weak, as compared to rat AHH. _Ambystoma_ AHH can readily participate in N-hydroxylation reactions which activate aromatic amine carcinogens, including the classical model substrate, 2-acetylaminofluorene and the environmental carcinogen, benzidine. This suggests that the salamander may prove to be a useful aquatic model organism for the study of chemical carcinogenesis.

Research on the use of feral populations of mollusks has born interesting results during FY 81. This research project carried out along the coast of Oregon by Mix, used indigenous populations of economically-important bivalve mollusks to detect and quantify environmental polycyclic aromatic hydrocarbons (PAH), including 11 compounds classified as carcinogens, 11 EPA Priority Pollutants and 11

Toxic Pollutants. Shellfish were also examined histologically to determine whether or not there was any association between the prevalence of cellular proliferative (perhaps neoplastic) disorders in these animals and the degree of environmental contamination, as determined by measuring PAH concentrations in their tissues. Total concentrations of 15 unsubstituted PAH were 30-60 µg/kg in shellfish from uncontaminated waters to greater than 1000 µg/kg in those from sites classified as contaminated. Cellular disorders were found in significant numbers in a mussel (Mytilus edulis) population with the highest PAH concentrations (average prevalence = 10%), while it rarely appeared in a second population with low concentrations. The correlation between the degree of PAH contamination and the prevalence of the disorders may be significant and warrants further investigation.

2. Biochemistry

A method was developed that allows the complete separation of all metabolites of BaP in one HPLC chromatogram. As reported last year in our studies with rats, glucuronosyl- and sulfo-transferase were induced by treatment with phenobarbital (PB), 3-methylcholanthrene (3-MC) and phenanthrene (PA). It has now been established that the induction of these transferases in the mullet is caused by 3-MC and in sea catfish by PB. The inductions were successful only after repeated injections at 16 and 30 days.

Since the microsomal fraction of liver cells contains enzymes other than the mixed-function oxidases (MFO), such as the glucuronosyl transferase (sulfo-transferase is in the supernate), steps were taken to establish their influence on the formation of products from the MFO reaction. First, a procedure was established to determine epoxide hydrolase (hydratase) activity and the inhibitor (1,2-epoxy-3,3,3-trichloropropane) was evaluated for its activity. Together with an inhibitor (D-glucaric acid 1,4-lactone) for the glucuronosyl transferase, attempts were made to establish some estimate of metabolite distribution with the glucuronosyl transferase present. We found that the lactone does not affect total inhibition of conjugation and apparently has little, if any, affect on the metabolism of BaP. The 1,2-epoxy-3,3,3-trichloropropane affects the metabolism of BaP and reduces by 75% the amount of metabolites formed. We are now in the process of ascertaining whether or not the metabolism was stopped at the first epoxides. [Schoor, Strength (CR809673), and Melius (CR809493)].

Efforts were made to study possible macromolecular phase transitions in DNA upon binding with metabolites of BaP. Both circular dichroism (CD) and optical rotatory dispersion (ORD), using salmon sperm DNA, reacted with 9-OH BaP and (t)-7,8-diol-9,10-epoxide, showed no spectral changes at 25°C. The effect of temperature has not yet been studied. While direct interference with the three dimensional structure of DNA at room temperature may not be a factor, a decrease of eight-fold in the relative fluorescence yield was shown in the case of the 9-OH and DNA. This could mean that loosely bound charge-transfer complexes can be found with interactions too weak to affect structural changes. We also found interesting correlations between fluorescent yield and isomeric positions of the twelve phenols, as well as unexplained, differentiated interactions between them and various MeOH/H_2O

mixtures. (Schoor).

VII. Significance to Biomedical Research and Program Needs of NCI and EPA

The following contributions to the needs of NCI and EPA were made during FY 81:

1. The responsiveness of the sheepshead minnow and the rainbow trout to the carcinogens benzidine and benzo(a)pyrene, respectively, was partially determined. Sheepshead minnows developed liver lesions resembling neoplasms, and the rainbow trout developed overt hepatocellular carcinoma. Both of these species have high potential to be used routinely as carcinogen assay organisms. The tiger salamander (Ambystoma sp.) is particularly interesting as an assay or indicator organism because of its cutaneous and liver enzyme responses to the PAH, perylene. The fact that this species has shown cutaneous tumor development in contaminated environments heightens its significance as a possible indicator species.
2. Field monitoring of polycyclic aromatic hydrocarbons and neoplastic diseases in mollusks in Oregon estuaries reveals a positive correlation between PAH's and disease in feral estuarine animals. This suggests the need to monitor routinely feral populations of wildlife for contaminants and possibly related diseases.
3. Biochemical and correlated structural responses of fish liver systems seem to be relatively similar to responses of mammals to certain carcinogens. Recent results show that the mixed function oxidases are induced in fish as well as the conjugating enzymes that aid in the detoxification and excretion of compounds such as B(a)P. This permits future comparative studies to determine if biochemical methods may be incorporated in early warning or sentinel monitoring projects with fish. The biochemical studies suggest that fish may serve as animal models in carcinogen (preneoplasia) studies to complement mammalian studies.

VIII. Proposed Course - Future Plans

We plan to pursue the following efforts in FY 82 in the project:

1. Continue and expand our use of fish carcinogen-assay systems by testing new compounds and, perhaps, by using critical life stages (embryos). Carcinogens found in the field monitoring study may also be tested against selected fish species in short term and longer term exposures. Improve diagnostic techniques for preneoplastic and neoplastic lesions in fish and shellfish, combining morphologic and pathologic methods with biochemical and clinical chemical methods.
2. Continue the field study of tumors in feral fish and shellfish populations (epizootiology); perhaps with the introduction of caged specimens into contaminated and clean waters for follow-up studies of disease development.
3. Continue and expand the biochemistry effort in the study of metabolism, conjugation, and excretion of carcinogens in fish and invertebrates. Studies will be initiated to examine the usefulness of biochemical determinations in small fish species used in the

carcinogen assay system. Baseline date will be gathered on silversides (Menidia) and sheepshead minnows (Cyprinodon variegatus). Examine the ways in which man may be exposed to carcinogens or their metabolites via the aquatic environment.

4. Explore the possibility of using a genetically controlled fish species (i.e., Rivulus sp.) in teratogenic and carcinogenic assay systems, and develop a chromosomal-cell culture technique utilizing an aquatic animal for developing a sister chromatid exchange test system for genotoxic agents.

IX. Date Contract Initiated and Period of Contract Planned

Initiated	Expiration Date
October 1978	September 30, 1984

X. Contractors Project Director

Dr. Henry F. Enos, Laboratory Director
Gulf Breeze, ERL, EPA

Project Officers for NCI or EPA

NCI:	Dr. Herman Kraybill
EPA/ORD:	Dr. Wayne Galbraith
Prinicipal Investigator:	Dr. John A. Couch, Gulf Breeze, ERL, EPA

XI. Cooperative Agreements Funded - Progress Reports FY 81

Title: Development of a Carcinogen Assay System Utilizing Estuarine Fishes
Principal Investigator: B.J. Martin, University of Southern Mississippi
Cooperative Agreement Number: CR806212
Project Officer: John A. Couch

During this period we have conducted or have in progress over 30 experiments in which Cyprinodon or Ictalurus are in some manner exposed to either benzidine dihydrochloride (BEN) or diethylnitrosamine (DENA). Our major efforts have been with BEN since we had preliminary evidence suggesting that we were likely to have more success with this compound and it seemed safer to work with BEN in our facilities.

1) Tumor Induction with Benzidine. Proliferative lesions have occurred in an experiment in which 50 female Cyprinodon were maintained in water contaminated at 12 day intervals with 1 ppm benzidine dihydrochloride. From day 166 to day 200, five individuals became moribund, were sacrificed and were discovered to have proliferative liver lesions. Although there appears to be some variation among specimens, all these lesions involve tubular proliferation, either bile ductules or vascular tubules. Efforts are underway to gain assistance in identifying these lesions from individuals with expertise in teleost oncology. Preliminary suggestions

are that these lesions could be hemangioendotheliomas, cholangiocarcinomas, or bile duct adenomas. A total of 17 fish was sacrificed and prepared for histological observation during this experiment. Examinations of these tissues are currently incomplete; however, to date, proliferative lesions have been observed only in liver tissue.

Repeat experiments are underway in an effort to confirm these results and similar experiments are being conducted in which the concentration of weekly contamination is 0.5 ppm. All these experiments are scheduled to be completed by the end of our project period.

2) *Pulse Exposures with Benzidine*. A common procedure for exposing rodents to carcinogens has been to place a toxic amount of the carcinogen in their water supply for a transient period after which they are allowed to recover and maintained through the latency period. This method of exposure has been a very effective way to induce clinical tumors. In an effort to duplicate this type of study, we initiated experiments in which *Cyprinodon* were exposed to 25 ppm, 40 ppm, or 50 ppm BEN until they were severely stressed (usually two to three weeks). After this exposure, the fish were allowed to recover and are being maintained in the laboratory. These experiments have been in progress for four months.

3) *Benzidine Feeding Experiments*. We have continued to have difficulty maintaining adequate numbers of live specimens for the necessary period of time if the fish are fed benzidine (1 gm/100 gm food) contaminated food. They are apparently stressed by this regime and significant numbers die during the course of the experiment. Currently, we have one experiment in progress in which 10 of 30 *Cyprinodon* are alive after almost five months of exposure. We plan to start experiments at a reduced level of food contamination in an effort to reduce the stress factor enough to be able to maintain fish for the required amount of time.

4) *Injection Experiments*. Efforts to conduct experiments in which *Cyprinodon* are injected with either BEN or DENA have continued to meet with difficulty. We have attempted to conduct these experiments with lower concentrations of carcinogen in the injection fluids; however, unacceptable rates of mortality have continued to occur. *Ictalurus* injected with 100 µl of 1% DENA survived for approximately four months; however, at that time, a mechanical problem caused the death of all the fish. This experiment *Ictalurus* is being repeated.

5) *Embryo Exposures*. During this year, we are making an extensive effort to study the total effects of BEN on developing *Cyprinodon* embryos. Two basic types of experiments are being conducted. Fertilized eggs are either being maintained in 1 ppm BEN throughout their development or they are being "pulsed" for a short period with a high concentration of BEN after which they are transfered to benzidine-free water. Organisms from these experiments are being studied by light and electron microscopy for possible effects of the contaminant.

6) *Aseptic Embryo Technique*. Previous efforts to develop primary cell cultures from early *Cyprinodon* embryos indicated the feasibility of maintaining these embryos for extended periods of time under aseptic conditions. The value of such a system for detailed studies of the effects of carcinogens at the molecular level is obvious. We have,

therefore, devised a procedure to sterilize fertilized eggs which are then maintained aseptically in tissue culture media. To date, we have demonstrated that embryos can remain viable under these conditions for at least 30 days. We are currently comparing the morphology of these embryos with embryos that have been maintained under normal "septic" conditions for the same period of time. Preliminary experiments are also underway in which the aseptic embryos are being maintained in media contaminated with 2.5 ppm BEN.

7) Static Embryo/Primary Cell Culture Experiments. Gnotobiotic embryos are being prepared according to the Linbro Plate Technique. We have spent the first part of the year perfecting this technique and are currently initiating experiments in which specimens in this system, containing a living embryo and a primary cell monolayer, are exposed to various levels of BEN. Initial results indicate that we should be able to maintain fish embryos in the system for at least 30 days. This should be sufficient time to produce transformed cells (mutilayered foci) of the type we have already produced with SHF-1 tissue culture cells.

8) Immunologic Studies. We are continuing to collect data concerning the quantity of t-lymphocytes in BEN-exposed and unexposed *Cyprinodon* by use of the previously developed Rosette Technique. Most of our efforts this year, however, have been devoted to the development of techniques for determining immunocompetence by direct quantitation of *Cyprinodon* immunoglobulins. The small size of this fish made it necessary to develop miniaturized procedures for quantitating serum proteins by electrophoresis. One can obtain ca. 10 to 20 µl of serum from an adult *Cyprinodon* and the agarose gel electrophoresis procedure developed requires only ca. 0.6 µl of serum. Thus, it is possible to run a number of replicates with the serum from each fish. Experiments to date indicate a definite difference in serum proteins between BEN-exposed and unexposed fish. Unexposed *Cyprinodon* produce distinct bands corresponding to albumen, alpha 1, alpha 2, beta and gamma globulins. After two weeks exposure to 1 ppm BEN, some of the fish had additional bands in the beta and gamma globulin regions and others had additional bands of alpha globulins. These differences seem more pronounced after three weeks exposure to BEN. Densitometer scans are being employed to more accurately quantitate these results; however, considerably more work will be required to provide enough data for meaningful analysis. As soon as antisera against whole *Cyprinodon* serum and immunoglobulins can be prepared, the study will be expanded to include immunoelectrophoretic techniques.

Title: Rainbow Trout: A Model for Environmental Carcinogenesis
Principal Investigator: Jerry D. Hendricks, Oregon State University
Cooperative Agreement Number: CR809344
Project Officer: John A. Couch

The dietary exposure of rainbow trout to three suspect environmental carcinogens, benzo(a)pyrene (BAP), Aroclor 1260, and toxaphene, has been in progress for 17 months. Random samples of 20 fish were taken from each duplicate group at 6 and 12 months to monitor tissue concentrations of these compounds and determine histopathological effects and the onset of neoplasia. No particular changes were noted at six months, but at 12 months, five of 33 fish from the BAP groups had either neoplastic (one hepatocellular carcinoma) or preneoplastic (four basophilic nodules) lesions of the liver. The carcinoma and one of the basophilic nodules were observed grossly, but the other nodules were discovered in random single sections from each liver. It is possible that more of these nodules could have been present in the livers. We believe these nodules are preneoplastic lesions that eventually develop into hepatocellular carcinomas. Thus, we are optimistic that a significant incidence of hepatocellular carcinomas will develop by the scheduled termination date of 9-28-81 (18 months after initiation of the carcinogen-containing diets). No tumors were observed at 12 months in the fish fed Aroclor 1260 and no sample was taken from the toxaphene fish in order to preserve 60 fish per group for the final sample. Mortalities have been low but persistent with this compound. Another group of trout has been receiving monthly intraperitoneal injections of 1 mg BAP in propylene glycol to assess the effect of BAP by this exposure route. Embryo exposures to BAP were not conducted, since the solubility of BAP in water is so low, adequate exposures were impossible.

The major significance of this work to date has been the limited positive response of rainbow trout to the carcinogenicity of BAP. If this response progresses as we now expect it to, it will constitute the first confirmed instance of an aquatic animal developing neoplasms due to BAP exposure. The dose level (1000 ppm in the diet) has been high, but we believe that is a highly significant finding.

Title: Causes of Papillomas on Fish Living in Chlorinated Sewage Effluent
Principal Investigator: John M. Grizzle
Cooperative Agreement Number: CR 807844010
Project Officer: John A. Couch

Adult black bullheads (*Ictalurus melas*) from the final oxidation pond of the Tuskegee, Alabama sewage treatment plant had a 70% prevalence of oral papillomas. The water in this 0.8-hectare pond is chlorinated effluent from the sewage treatment plant. The various experiments in this project were designed to determine the etiology of these papillomas.

Exposure of fish to water in the final oxidation pond. Juvenile black bullheads, yellow bullheads (*Ictalurus natalis*), and channel catfish (*Ictalurus punctatus*) were placed in 1-m^3 cages that were allowed to rest on the bottom (sinking cages) or were suspended off of the bottom by floats (floating cages). Cages were placed in three locations in the oxidation pond: near the inlet (inlet A), 60 meters from the inlet (inlet B), and near the outlet. Caged control fish were kept in a 0.04-hectare pond. Fish were fed small quantities (3% of body weight twice a week) of a commercial catfish ration. Three of the 200 black bullheads in 2 cages at the inlet A location survived for 52 days; one survivor had grossly visible, focal hyperplasia of the oral mucosa. Additional descriptions of this and similar lesions found on caged fish are given below. At inlet B location, 50% of the 12 surviving black bullheads in one cage had oral lesions after 43 days. These lesions healed after 109 days. In cages near the outlet, 91% of black bullheads had oral lesions after 168 days. Most of these lesions healed, and 9% had oral lesions 234 and 256 days after stocking. Channel catfish exposed for 189 days and yellow bullheads exposed for 85 days had frequencies of oral lesions similar to controls. These fish will be maintained in cages for additional exposure to determine if papillomas will develop.

The percentage of black bullheads with oral mucosa hyperplasia was similar in the sinking and floating cages near the outlet. Too few fish

Summary of 6-month sample of rainbow trout exposed to
dietary benzo(a)pyrene, Aroclor 1260 and toxaphene

Compound & level	Body weight (g)	$\frac{\text{Liver wt.}}{\text{Body wt.}} \times 100$	Cumulative mortalities (1-6 mo.)
Benzo(a)pyrene (100 ppm)	42	1.20	1
Aroclor 1260 (500 ppm)	47	.93	2
Toxaphene (100/50 ppm)*	37	---	64
Control	50	1.13	7

*Diet changes from 100 ppm to 50 ppm after 2 months.

Summary of 12-month sample of rainbow trout exposed to
dietary benzo(a)pyrene, Aroclor 1260 and toxaphene

Compound & level	Body weight (g)	$\frac{\text{Liver wt.}}{\text{Body wt.}} \times 100$	Cumulative mortalities (6-12 mo.)	Tumor Incidence*
Benzo(a)pyrene (1000 ppm)	158	0.83	7	5/33
Aroclor 1260 (500 ppm)	194	0.83	0	0/40
Toxaphene (50 ppm)	164	---	13	---
Control	196	0.73	1	0/39

*Included 1 hepatocellular carcinoma and 4 basophilic nodules.

survived to compare cage types near the inlet. The lesions on fish in the floating cages indicated that contact with the sediment or ingestion of benthic food organisms was not necessary for development of the lesions.

The control fish of all three species had a low prevalence (2-14%) of oral lesions that appear similar to those in the experimental pond. The cause of these lesions is unknown.

Histology of tumors and related lesions of fish in the oxidation pond. Histologically, the papillomas on feral black bullheads from the oxidation pond consisted of a hyperplasic mucosa covering papillae of submucosa (Grizzle et al. 1981). Some of the epithelial cells of the papilloma contained inclusions that were eosinophilic, PAS-positive, and Feulgen-positive. The most distinctive feature of the tumors was their uniform location in the mouth fornices.

The grossly visible, oral mucosa lesions in caged black bullheads were consistently located in the same location as the tumors on feral black bullheads from the oxidation pond. These lesions had a mucosa approximately 600 µm thick, compared to a normal thickness of 20-100 µm, over a hypercellular submucosa that was more vascular and up to ten times thicker than normal. Most of the epithelium in the lesions consisted of stellate-shaped cells like those in the papillomas of adult black bullheads. Goblet cells and alarm substance cells were less common in the epithelium of hyperplasic lesions than in normal oral mucosa, and inclusion bodies were not present.

Ultrastructure of tumors and related lesions in fish from the oxidation pond. Most of the epithelial cells in the papillomas examined were separated by an intercellular space, except for small zones where cells were held together by desmosomes. In early studies, electron-dense cells contained numerous 35nm particles not seen in other cells. In the spongy zone of papillomas examined recently, these electron-dense cells were not present. Future work will concentrate on the peripheral part of the papilloma that contains inclusions in compact, spherical cells.

Thickened oral mucosa from caged black bullheads in the oxidation pond and normal oral mucosa from adult and juvenile black bullheads have been embedded for electron microscopy. Sections of this material will be compared to sections of black bullhead papillomas.

Analysis of water from the oxidation pond. Concentrations of zinc, copper, cadmium, iron, and manganese in the oxidation pond were low, and pesticides in the PCB and organophosphate groups were not detectable in the 100 ng/l range. Nitrogen and phosphorus organic residues were detected in the 100 ng/l range, but were not identified. Chloroform and bromodichloromethane were present in water from the sewage treatment plant, but in low concentrations. Bromoform and chlorodibromomethane, with a detection limit of less than 1.0 µg/l, were not detectable. The highest concentrations of chloroform (14 µg/l) and bromodichloromethane (0.9 µg/l) were at the inlet to the final oxidation pond, but the chloroform concentrations were similar at the outlet (10-12.5 µg/l). A water sample taken before chlorination during the sewage treatment process had 11 µg/l chloroform, which indicated that the chloroform concentration was being changed little by the chlorination procedure.

Mutagenicity testing of the sewage pond water. Two different organic-compound concentrates of the water were prepared. An acidic fraction was obtained by acidifying the water to pH 1.0 and extracting the organic matter three times with a mixture of 25% ether and 75% hexane. A basic fraction was prepared by adjusting the pH to 12-13 and extracting the organic matter with 25% ether and 75% hexane. Both extracted solutions were dried at 30-40°C. Concentrates were tested for mutagenicity as described by Ames et al. (1975). Dimethylsulfoxide (DMSO) solutions of the water extracts were tested at various dilutions with and without Aroclor-induced rat liver enzymes (S-9) in *Salmonella typhimurium* tester strains TA-98 and TA-100 with 5 replications per test. Positive controls had 2-10 μg of benzo(a)pyrene with S-9, and negative controls had DMSO, S-9, or hexane.

The acidic fraction of sewage pond water, in the presence of S-9 enzymes, caused a significant increase in revertants for both tester strains. Results for the basic fraction were negative.

Transmission of tumors by cell-free tumor homogenates. A papilloma from a black bullhead taken from the oxidation pond was homogenized in Hanks' balanced salt solution (HBSS), passed through a 0.45 μm filter, and injected subcutaneously into the mouth fornix of three adult black bullheads. Control bullheads were injected with filtered HBSS. Each fish was kept in a separate 40-l glass aquarium and examined at approximately monthly intervals. No lesions had developed after 14 months, and the experiment was terminated.

Four papillomas were homogenized in HBSS, filtered through a 0.45 μm membrane, and lyophilized in separate ampules to concentrate the homogenate. One-year-old black bullheads were acclimated to 40-l glass aquaria, one fish per aquarium. During May 1981, 16 fish were injected with one of the homogenized papillomas and five control fish were injected with filtered HBSS. No lesions were present in the injected fish after 110 days. These fish will be observed for several more months for development of lesions.

Hepatic enzymes in black bullheads and silver carp. Silver carp (*Hypophthalmicthys molitrix*) obtained from a control pond and put into the oxidation pond for 3 to 4.5 months had a 22% increase in the liver/body weight ratio (Table 1). The liver/body weight ratio was 3.4 times higher for feral black bullheads from the oxidation ponds than for controls. Even during a chronic epizootic, moribund black bullheads that were not feeding as well as the healthy fish had a liver/body ratio 2.5 times higher than controls. Although the increased liver size may be due in part to the additional food available in the oxidation pond, the enlarged livers in the moribund black bullheads indicate that chemicals or infectious disease affected the size of the liver.

The increase in HGOT and decrease in the ChE serve as simultaneous indicators of hepatic dysfunction (Table 1). The Cyt P_{450} (or P_{420}) and microsomal MFO-related enzymes are presented in Table 2. None of the cytochromes was observed in the control black bullheads, and Cyt P_{450} was only observed in male black bullheads without tumors from the oxidation pond. This could be due to denaturing of the cytochromes from 450 nm to 420 nm because the microsomes were not glycerol-stabilized. Even so, the Cyt P_{420} gives an indirect indication of Cyt P_{450} induction. The NADPH-cyt cR was slightly increased by 27% and 18% in the male and female black bullheads, respectively. The change in the silver carp NADPH-cyt cR was not significant. The AHH was increased by 1.6 times and 3.0 times in the male and female black bullheads, and 2.2 times in the silver carp when compared to the corresponding controls. However, in the total BaP metabolism the differences in the control and oxidation-pond fish were not significant.

HPLC separation indicated that the 3-hydroxy-BaP and 7,8-diol-BaP were the major products and to a lesser extent 9,10-diol-BaP, 9-hydroxy-BaP and 1-hydroxy-BaP. These metabolites were identified by comparison with the retention time of standards. Preliminary data indicated that epoxide hydrase was present in bullheads and silver carp, and that for the corresponding species, oxidation-pond fish had higher levels of activity than control fish.

Table 1. Liver size and hepatic enzymes of silver carp and black bullheads from the final oxidation pond and a control pond.

	Silver carp[a] (control pond)	Silver carp[a] (oxidation pond)	Bullhead (control pond)	Bullhead (oxidation pond; healthy)		Bullhead (oxidation pond: moribund)[d]	
				T[b]	NT[c]	T[b]	NT[c]
Liver/Body ratio (g/kg)	9.4	11.5	13.3	48.1	41.8	32.3	35.0
HGOT[e] (nmole/mg/min)	---	----	4.55	13.6	15.9	8.55	2.85
Cholinesterase (nmole/mg/min)	---	----	1.56	0.58	0.92	0.60	0.88

a Silver carp were in the sewage pond for 3 to 4.5 months.
b Tumor.
c No Tumor.
d Moribund fish had been observed in the pond for about 2 weeks and did not resist capture.
e Hepatic glutamic oxaloacetic transaminase.

Table 2. Cyt P_{450} (or P_{420}) and microsomal MFO-related enzymes of black bullheads and silver carp from the final oxidation pond and control pond.

	Cyt P (nmole/mg)	Cyt P (nmole/mg)	NADPH-Cyt cR (nmole/mg/min	AHH (pmole/ mg/min)	Benzo(a)pyrene metabolism[c]
Black bullheads					
C -male	------	------	17.7	34.4	57
NT -male	0.21	0.61	24.8	62.1	50
T -male	------	1.60	20.3	50.0	53
C-female	------	------	16.9	25.2	44
NT-female	------	2.05	18.1	75.8	49
T-female	------	5.8	21.9	78.0	51
Silver carp					
Control pond	------	0.15	13.8	44.1	58
Oxidation pond	------	------	12.8	97.0	65

[a] Average of 2 to 4 runs

[b] Measured according to the 3-hydroxylated B(a)P.

[c] 1 mg of microsomal protein was incubated with 20 nmol B(a)P for 30 min at 37°C, and under red light illumination. Percentage estimated from decrease in BaP.

[d] control pond;

[e] fish without tumor from oxidation pond.

[f] fish with tumor from oxidation pond;

[g] Silver carp were put in the oxidation pond for 3 to 4.5 months.

Title: Environmental Perylene as a Possible Carcinogen
Principal Investigator: Robert S. Anderson and Francis L. Rose
Cooperative Agreement Number: CR807740
Project Officer: John A. Couch

We have now started our studies to determine if the tiger salamander (Albystoma tigrinum) is a useful animal model for studying chemical carcinogenesis and for testing putative aquatic carcinogens. We are emphasizing perylene (P) because of the known association of elevated concentrations of environmental P with cutaneous neoplasia in this species, and because of its reported high mutagenicity in bacterial assay. The program is divided into two distinct areas of study. 1) Studies of P metabolism by Ambystoma are carried out at the Sloan-Kettering Institute. All known polycyclic aromatic hydrocarbon (PAH) carcinogens require metabolic activation for optimal biological activity; no studies have previously been made of P metabolism in any species. 2) Studies of the carcinogenicity of P are carried out at Texas Tech University. P was administered i.p. to young neotenes, dosage was MTD (maximal tolerated dose). The possible carcinogenic effect of P with benzo(a)pyrene is being determined. P will also be studied in a classical two-stage skin carcinogenesis assay, using phorbol myristate acetate as the promoting agent.

Basis for ^{3}H-P-Hydroxylase Assay. The assay is based on the aryl hydrocarbon hydroxylase (AHH) assay of Abramson and Hutton (Cancer Res. 35: 23-29, 1975), which quantifies the generation of NaOH-soluble and aqueous phase metabolites of radiolabeled benzo(a)pyrene (BaP). This assay has been shown to be more sensitive than the fluorescence technique, which measures mainly 3-OH BaP and 9-OH BaP, in that it can also measure dihydrodiols, metabolic conjugates, and metabolites covalently bound to proteins. In this assay, labeled BaP is incubated, under optimal conditions, with an appropriate AHH source, such as hepatic microsomes. The reaction is terminated and the reaction mixture is extracted with hexane; phenolic derivatives and most of the dihydrodiols are extracted from the hexane with NaOH. The aqueous portion of the hexane-extracted reaction mixture also contains a number of labeled metabolites which may be quantified. The organic phase contains the parent compound and BaP quinones.

We have shown that this extraction technique is useful in quantifying the metabolism of P, which is an isomer of BaP.

Preparation of ^{3}H P Working Stock. A sample of Aldrich gold label perylene was immediately sent to ICN Radioisotope Division for custom tritium labeling by catalytic exchange. Upon analysis by HPLC, it was evident that the majority of the radioactivity was associated with perylene; several unidentified, nonperylene peaks were also present. However, we felt it desirable to increase the radiopurity of the perylene before trying to use it in our assays. It was possible to purify the sample by the use of Sep-Pak (Waters Associates) containing μBondapak C_{18}, thus obviating the need for an expensive preparative HPLC column. Approximately 90% radiochemical purity was obtained after this treatment. It is necessary to repurify ^{3}H-P frequently because of its gradual decomposition during storage.

^{3}H-P-OHase Assay Using "Mutazyme". Before undertaking studies of perylene metabolism in Ambystoma, we used a defined source of mammalian AHH to ascertain that our methods for quantifying BaP metabolism could be used in comparable studies of perylene. Mutazyme (Meloy Labs) is a commercial AHH source designed for use in the Ames bacterial mutagenicity test. It is a lyophilized S-9 preparation containing hepatic microsomes from rates previously induced with Aroclor 1254. In other studies, we showed that Mutazyme can provide an excellent AHH source for *in vitro* BaP metabolism. Therefore, we compared ^{14}C BaP and ^{3}H P metabolism under identical assay conditions, as mediated by Mutazyme.

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^{14}C-BaP and ^{3}H-P Metabolism by Mutazyme

	NaOH-soluble metabolites	H_2O-soluble metablites	Total
^{14}C-BAP	2.076 ± 0.626	2.692 ± 0.728	4.768 ± 0.623
^{3}H-P	1.926 ± 0.384	3.571 ± 1.226	5.497 ± 1.049

Metabolites expressed as nmole/mg protein/10 min; n = 5

Clearly perylene was metabolized to the same extent as BaP in this system; more water-phase metabolites seemed to be generated than NaOH-soluble products, particularly in the case of perylene. The extraction procedures developed for BaP metabolites appeared satisfactory for P.

^{14}C-BP Metabolism by *Ambystoma* Site-Frozen Hepatic Samples.

Preliminary studies designed to aid in establishing optimal *in vitro* conditions for *Ambystoma* AHH activity were carried out with the classical substrate, BaP. The main reason for selecting this substrate was that a suitable sample of labeled perylene was not available for many months.

Ambystoma liver samples were excised and rapidly frozen at the site in Texas and subsequently sent to this laboratory packed in dry ice. Samples were stored at -80° until used; aliquots were homogenized and S-9 prepared as required. *Ambystoma* hepatic samples were incubated with ^{14}C BaP for 30 min; AHH activity was determined for homogenates and S-9 preparations at temperatures from 20-37°, in presence or absence of NADPH or an NADPH-generating system. Enzyme activity was low and variable; nevertheless, some useful information about optimal conditions was produced. Both homogenates and S-9 preparations showed activity which appeared to be NADPH-dependent, although no clear differences were seen between the NADPH-generating system and NADPH alone in the medium. AHH activity appeared to be greatest at an incubation temperature of 37°C. If the protein concentration of either homogenate or S-9 added to the reaction vial exceeded about 1 mg, the generation of metabolites was reduced. The maximal total ^{14}C BaP metabolite production recorded for either homogenate or S-9 preparations from these prefrozen liver samples ranged from about 0.1-0.2 nmole/mg protein/30 min. In most cases, the majority of BaP metabolites were in the water phase.

Perylene Metabolism by Fresh *Ambystoma* Liver.

A. Uninduced animals.

Salamanders were sent to this lab by special courier within a few days of collection from the Reese Air Force Base sewage lagoon. They were usually in excellent condition upon arrival, and were subsequently housed in 50-gallon holding tanks. Water was changed frequently and was continuously aerated and charcoal-filtered.

Liver homogenates and S-9 fractions were tested for BaP- and P-OHase activity from two uninduced females. Benzo(a)pyrene hydroxylase activity was present in homogenates (X = 0.051 nmoles/mg/30 min) and S-9 (X = 0.462 nmoles/mg/30 min), but P-OHase was only detected in one homogenate sample and not measured in the S-9. However, in two uninduced males both BaP-OHase and P-OHase activity was observed in S-9 preparation. Under optimal conditions, hepatic S-9 from uninduced males had P-OHase activity of about 0.1 nmoles/mg/30 min. B. Induced animals

Since preliminary studies with untreated Ambystoma had indicated that AHH activity was low, and possibly sex-dependent, we attempted to increase its activity by induction. Two well-known inducers of PAH metabolism were used: the polychlorinated biphenyl, Aroclor 1254 (500 mg/kg, i.p. 96 hr before sacrifice), or 3-methylcholanthrene (50 mg/kg, i.p., 48 hr before sacrifice). Also, since there were apparent differences in the ability of salamander enzymes to metabolize P and BaP, for the purpose of this study, we thought it advisable to concentrate on P metabolism exclusively.

Both Aroclor and MC were shown to be effective inducers of P-OHase activity. The induction obtained was not as dramatic as that reported in mammals; however activity was consistently measured in hepatic homogenates and S-9 fractions from both males and females. There is no clear indication that induced males have more active P-OHase than induced females.

Optimal reaction conditions were determined by varying incubation time, temperature, cofactors, enzyme concentration, etc. The results indicated that the reaction proceeds best at 37°C for 30 min in the presence of an NADPH-generating system, rather than NADPH alone, and with no more than 50 µl homogenate or 100 µl S-9 (about 1 mg protein). When run under these conditions, the hepatic homogenate P-OHase activity in Aroclor-induced Ambystoma was 0.468 ± 0.179 (n = 8) nmoles metabolites produced/mg protein/30 min; activity in liver homogenates from MC-induced animals was 0.333 ± 0.101 (n=7) nmoles/mg/30 min.

Perylene Metabolism by Fresh Ambystoma Skin. Since the vast majority of tumors in salamander populations in the Reese AFB lagoon are cutaneous, and since the integument of the animals is in constant contact with perylene-containing water and sludge, we thought that it would be interesting to measure AHH activity in the skin. P-OHase activity of S-9 was not significantly different from that of homogenates; Aroclor and MC induction were comparable. Dermal homogenates from Aroclor-induced animals gave ^{3}H-Pase activity of 0.258 ± 0.130 nmoles/mg/30 min (n = 8); similar preparation from MC-induced salamanders gave 0.344 ± 0.129 nmoles/mg/30 min (n = 11).

Generation of Mutagenic Perylene Metabolites. Perylene, when activated with Mutazyme, was tested against the standard Ames bacterial tester strains. The best responses were obtained with TA 1537, although some response was seen with TA 98. Perylene was shown not to be a direct mutagen, but must be activated like BaP. We have not yet been able to obtain mutagenesis using Ambystoma AHH and perylene; weak responses to BaP have been produced. Interestingly, we showed that Ambystoma AHH can activate 2-aminofluorene. Aromatic amines, such as benzidine, are carcinogenic pollutants of the aquatic environment; Ambystoma may prove to be a useful model to their study.

Studying the Carcinogenic Effect of P on Ambystoma. An aquatic system to house large numbers of experimental animals has been developed. This system includes 50 sixteen-liter, 10 one hundred-liter, and 2 fiberglass "living stream" aquaria. A continuous flow system is now in use that allows the water to turn over every 24 hours. In-line charcoal filters remove copper which is highly toxic to younger animals. PAH in the effluents is removed by adsorption on charcoal filters. The quantity and route of administration used preclude the likelihood of the presence of significant amounts of PAH or metabolites in the water.

Feeding regimens have been worked out and larvae are doing well on a diet of beef heart, minnows, and beef liver given on successive days. This step is necessary in order to permit long-term maintenance experiments that will not be altered by spontaneous metamorphosis.

Groups of at least 20 neotenes have been injected i.p. with perylene, benzo(a)pyrene, perylene + benzo(a)pyrene, or the injection vehicle. All groups have been maintained in apparent good health for several months. This study will provide data on the carcinogenicity of the putative carcinogen P, the carcinogenicity of the well-known oncogen BaP, and possible cocarcinogenic effects.

Title: Carcinogens and Neoplasia in Indigenous Populations of Aquatic Organisms
Principal Investigator: Michael C. Mix
Cooperative Agreement Number: CR808000
Project Officer: John A. Couch

Further refinements were made in the HPLC analytical procedures developed to identify and quantify unsubstituted PAH isomers. Concentrations of fifteen PAH's including phenanthrene, fluoranthene, pyrene, benzo(a)phenanthrene, triphenylene, benzo(a)anthracene, chrysene, benzo(b)fluoranthrene, benzo(k) fluroanthene, dibenz(a,c)anthracene, benzo(a)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene, ideno(1,2,3-c,d)pyrene and coronene were measured in shellfish monitors. The method resolved most members of the benzpyrene group. Perylene was not identified because it did not adsorb UV light at the wavelength used, and benzo(j)fluoranthene and benzo(e)pyrene could not be separated.

Baseline concentrations of PAH's in indigenous bivalve mollusks used as biomonitors reflected the degree of human onshore activity at the various sample sites and, presumably, the level of water contamination. PAH concentrations in shellfish from relatively pristine areas ranged from 30-60 μg/kg while those from industrialized areas contained 500-1500 μg/kg (Table 1).

The data collected during this study indicate that clam species, in addition to *M. edulis*, make excellent monitors for detecting and measuring PAH's in estuaries.

A major attempt was made to identify and evaluate quantitative and qualitative relationships between individual PAH's and between PAH's and their concentrations in bivalve mollusks. The results indicate that a significant potential exists for developing predictive models for PAH's in aqueous environments and their concentrations in certain seafood products. Some of the relevant findings and conclusions, based on thorough statistical analyses of

Table 1. RANGE OF PAH CONCENTRATIONS IN SHELLFISH MONITORS FROM VARIOUS SITES IN THREE OREGON BAYS.

BAY	SITE	SPECIES	PAH CON. (μg/kg)	RELATIVE DEGREE OF INDUSTRIALIZATION
Tillamook	TIM	*Mytilus edulis*	40-60	±
Tillamook	TSS	*Mya arenaria*	30-60	±
Tillamook	TBS	*Crassostrea gigas*	35-45	±
Yaquina	Y140	*C. gigas*	30-45	±
Coos	C3S	*M. arenaria*	70-90	±
Coos	C11G	*Tresus capax*	30-110	±
Coos	CSS	*M. arenaria*	480-650	+++
Yaquina	Y1M	*M. edulis*	140-440	+
Yaquina	Y2M	*M. edulis*	675-1325	++++

the data from these studies are summarized below.

1. Quantities of a single PAH present in shellfish cannot be used to predict total PAH.

2. For each site, different independent variables (individual PAH's) were identified and used to predict total PAH's in bivalve mollusks. It may be possible to identify site-specific independent variables after a suitable sampling period and subsequently to measure only those variables for an adequate assessment of total PAH's. Complete analyses could perhaps be made periodically to confirm the continuing validity of the established relationship; deviations may indicate new sources of contamination. Such an approach may result in considerable cost reduction for long-term monitoring programs.

3. Benzo(a)pyrene was not a significant variable for predicting total PAH's at any site. Thus, the concept that BAP can be used as an index of PAH contamination was not supported by the results of this study. From this and other studies, it seems that the use of BAP for making decisions about the quantities and presence or absence of other PAH's should be abandoned or modified.

4. While it was established that quantitative predictions about total PAH's could not be made on the basis of individual PAH measurements, the result of the analyses suggest that certain qualitative relationships existed which may permit first approximations of individual PAH concentrations. In general, there were no significant differences between individual PAH's with 4 rings, or between those with 5,6 or 7 rings. Phenanthrene, a 3 ring compound, differed significantly from other PAH's. Thus, detection of a certain quantity of PYR, for example, suggested that a similar quantity of BCP, TRI and BAA was present; measurement of an individual PAH concentration for any 5-,6- or 7-ring PAH indicated that approximately the same concentration would be found for any other unsubstituted PAH with 5-7 rings. It would be productive to conduct these kinds of analyses for PAH data collected during future studies and from other, established biological monitoring programs. Confirmation of the relationships identified during this research may eventually lead to a simplified monitoring approach and result in considerable cost reductions.

Statistical analyses revealed an empirical relationship between individual PAH concentrations and their respective solubilities. The concentrations in shellfish were greater for the PAH isomer which had the higher solubility in water. This finding contrasts with the observation that organic/water (e.g. octanol/water) partition coefficients show an inverse relation to water solubility. Because the concentration in the organic phase (shellfish, in this study), C_O, is equal to the product of the partition coefficient (K) and concentrations in water (C_W), the data suggest that the ratio of the PAH concentrations in water would have to be generally greater than the ratio of their reciprocal partition coefficients or their water solubilities. Direct measurements of PAH concentrations in seawater will be necessary to confirm whether the uptake of PAH's by shellfish can be represented by a simple partition process.

Data from studies of BAP in *M. edulis* suggest that the depuration rate for this compound was exponential with a half-life of 8-10 days, while uptake was linear. Routine depuration procedures in which shellfish are placed in clean seawater for 24 hours would have little effect in reducing BAP concentrations. Gametogenesis and/or incorporation of BAP, and presumably other lipophilic PAH's into the gonad do not appear to be directly responsible for seasonal increases of BAP in mussels during winter-spring. BAP storage occurred primarily in the somatic tissues, compared to the gonad, even during the spring spawning period.

Different populations of shellfish were examined histologically for the presence of cellular proliferative disorders. Clams from Coos Bay and mussels from Tillamook Bay were not found with the large abnormal cells that characterize the conditions. The disorder was present in a significant number (mean prevalence = 10%) of Yaquina Bay mussels with the highest concentration of PAH measured in this study while it rarely appeared in a second population at a "clean" site across the bay. The correlation between the degree of PAH contamination and the prevalence of the cellular disorders may be significant, but no cause-effect relationship has been established. It remains to be determined if carcinogenic metabolites can be formed by this species. If bivalves are not subject to PAH-induced carcinogenesis, and the presence of atypical cells is related to a neoplastic process, then other causative agents must be responsible.

Assuming the condition is analogous to neoplasia, it seems evident that this disorder in M. edulis had great potential for serving as a model for studying cancer-like diseases in an invertebrate. The cells have many characteristics in common with malignant conditions in mammals and affected mussels can be obtained easily and on a regular seasonal basis by procedures developed during this study. Considerable future effort should be directed towards further characterizing the cells, attempting to establish culture techniques suitable for maintaining and growing the cells, and identifying the causal agent(s).

The following studies have been initiated during the last 6 months, but are not yet completed:

1. measuring baseline levels of arsenic, cadmium and nickel in indigenous bivalve mollusks and other monitoring species;
2. determining if bivalve mollusks from heavily contaminated and relatively uncontaminated environments, as indicated by PAH tissue concentrations, differ in their ability to metabolize PAH's.
3. refining procedures used in employing populations of M. edulis as biomonitors for carcinogens in bays or estuaries;
4. determining if viruses are associated with the abnormal cellular disorders in M. edulis;
5. further characterization of the abnormal M. edulis cells, using scanning electron microscopy;
6. attempts to culture the abnormal, large M. edulis cells.

Title: Oxidation and Conjugation of Carcinogenic Hydrocarbons in Marine Animals
Principal Investigator: D.R. Strength, Auburn University
Cooperative Agreement Number: CR806673
Project Officer: W. Peter Schoor

Polycyclic aromatic hydrocarbons (PAH) are oxidized to epoxides, dihydrodiols and phenols. The resultant oxidation products may be mutagenic and carcinogenic. Conjugation with glucuronic acid or sulfate can occur and may diminish the cytotoxic effects of oxidation products and facilitate excretion by animals. Phenobarbital (PB), napthol (NO), phenolphthalein (PT), and selected PAH compounds (naphthalene, phenanthrene (PA), benzo(a)pyrene (BaP), methylcholanthrene (3MC) were fed or injected into rats, mullet, sea catfish, and killifish to ascertain the effects upon the enzymes for conjugation of oxidation products.

UDP-glucuronosyl transferase and sulfotransferase were significantly increased in livers of rats given PB, PT, PA, BaP or 3MC orally for 10 to 14 days. The injection of PB or 3MC into rats, sea catfish or mullet on a long-term schedule resulted in increase in liver size, and an increase in UDP-glucuronosyl transferase and sulfotransferase in livers of all three animal species. Age and sex of rats affected induction of the two enzymes in rats. Induction in fish was successful only after multiple injections and 16 to 30 days.

Assays for the 3-steps involved in sulfotransferase determinations were investigated and induction of the sulfotransferase (third stage) was demonstrated. Selected hydroxy compounds were conjugated with sulfate from ^{35}S-PAPS and ^{35}S-SO$_4$. The conjugated, radioactive sulfate esters were separated by thin-layer chromatography from respective ^{35}S-sulfate donors.

Title: Metabolism of Polyaromatic Hydrocarbons in Marine Organisms
Principal Investigator: Paul Melius
Cooperative Agreement Number: CR809493
Project Officer: W. Peter Schoor

We have applied the procedure for assay of 1,2-diol by indirect atomic absorption spectroscopy of digested lead periodate (Anal. Chem. 58, 602, 1980), and have developed two enzymatic assays of epoxide hydrolase (E.C.3.3.2.3.) in *Tilapia aurea* and rabbit. 1,2-diol produced by the action of the epoxide hydrolase on styrene oxide was measured by precipitation as the insoluble lead salt, which was then redissolved in nitric acid and measured by atomic absorption spectroscopy (Anal. Letters 14 (BS), 311 (1981). The difference between the initial amount of periodate and the final amount is equivalent to the amount of styrene oxide, which is the synthetic substrate for the epoxide hydrolase.

We found that trans-stilbene oxide (TSO) induced epoxide hydrolase activity in the rabbit and *Tilapia*, whereas Aroclor 1254 caused only a small increase in the epoxide hydrolase in the *Tilapia*. Both epoxide hydrolase activities were inhibited by 1,2-epoxy-3,3,3-trichloropropane (TCPO).

When TSO-treated *Tilapia* were used for analyses of changes of cytochrome P-450, no peak was observed at 450 or 420 nm by the Omura and Sato procedure. However, a peak was observed at 432 nm (Bull. Environ. Contam. Toxicol. 26: 801, 1981). Similar results have been reported by Fukami in the carp and also by Chambers and Yarbrough. An increase of cytochrome P-438 was observed with 350 mg TSO/kg treatment in *Tilapia*.

No significant increases of NADPH cytochrome-C reductase or aminopyrene

N-demethylase were observed at 200 mg TSO/kg in *Tilapia*. However, a 50% decrease in liver cholinesterase and increases in glutamate-pyruvate (25%) and glutamate-oxaloacetate (300%) transaminases indicated a cytotoxic effect in the liver of *Tilapia.*

XII. Publications, Reports and Abstracts from Program - Cumulative list (1978 - 1981)

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PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

Friday Afternoon, September 11

DISCUSSION GROUP

GROUP A - EPIDEMIOLOGY

Discussion Leader:
Dr. Robert Spirtas
National Cancer Institute

DR. SPIRTAS: My name is Bob Spirtas. I am with the National Cancer Institute. We have a charge today to try to, as best as possible, look at new methods, gaps, which endeavors or studies or information resources are missing, and which areas of research should be phased out.

I have looked at the previous notes from yesterday's meeting and see some ideas for studies. As a start, I will indicate my interests, and ask everybody if they will stand up and introduce themselves and give us a minute or so of background on the sorts of studies they are doing, and what they would be interested in talking about. And as people come in I will ask them to introduce themselves so that we can have an informal session.

I will start off by saying that most of my studies are in the occupational cancer area and that I have been doing both cohort and case-control studies. Further, I am interested in latent periods, and in gathering enough data in large enough studies to produce meaningful results. Could I ask you to start please? Stand up and introduce yourself and tell us what your interests are.

MS. KRAFT: I am Diane Kraft. I am with the Department of Labor, Mine Safety and Health Administration. I am an industrial hygienist with the metal and non-metal mines. We are currently interested in some studies carried out with nuisance particulates, specifically, potash and salt dust. There are many other areas that we are interested in but that is my area now that I would like to see further study in.

MR. FOUNTOS: My name is Barry Fountos. I work for OSHA as an epidemiologist in the Directorate of Health Standards Programs. For the past two years I have been working on heavy metals, particularly concentrating on human evidence of carcinogenicity in workers exposed to nickel compounds. Right now I can say that there isn't a lot of active work going on that I know of, other than some recent studies submitted from the nickel industry that so far have been non-positive as indicated by the authors. Unfortunately, I feel that quite a few of them suffer from an insufficient number of workers so that there is inadequate power to detect cancer at sites that you would expect cancer to develop. This precludes the ability to draw definite conclusions on the adverse health effects of nickel for which these studies were designed to detect.

DR. SPIRTAS: Thank you. Linda?

MS. ANDERSON: I am Linda Anderson with Cancer Communications, as a science writer there. So I am just here to try to keep up in the area.

DR. SPIRTAS: Thank you. Ken?

DR. BRIDBORD: I think I spoke my piece yesterday.

DR. SPIRTAS: Well, there may be people here who don't know you, or there may be someone who hasn't heard your reputation, let me put it that way. It is unlikely but come on up and introduce yourself. Just introduce yourself and explain what your interests are so that we can try to synthesize what we might talk about in the next few minutes.

DR. BRIDBORD: Oh, okay. I am Ken Bridbord with NIOSH. My background is as a physician and epidemiologist; and on occasion I have been involved in some of the discussions on cancer and contributing causes. One of the important things I believe in is that we are probably dealing with multiple contributing causes, not single things acting independently, and that people are too quick to assume that because relationship is found with one situation that that describes the total picture. I think we should just keep a broad perspective.

DR. SPIRTAS: Thank you. George?

DR. BURTON: I am Dr. George J. Burton, Environmental Epidemiology, National Cancer Institute. I am the Executive Secretary of the Evaluation Group that evaluates contract proposals for award, and have also been involved for the past year in monitoring tracing of subjects who are being studied in environmental epidemiologic studies on cancer.

Our experience has shown that there is considerable variation among organizations in tracing effectiveness. Occupational studies involve follow-up of subjects retrospectively, with attempts to determine, years after exposure to suspect carcinogenic substances, where subjects are located, and the present state of their health, if living. Specifically, the tracing organization ascertains their last known or current address, and, if they have died, the exact date of death along with the name of the town or city and state where death occurred.

We found that very often tracing success depends on the ingenuity of the personnel doing the tracing. This cannot always be ascertained readily from an organization's claim in a contract proposal as to how expert they are and how much experience they have had in tracing persons on whom little information is now known. If the subject is still alive and has been located, only the Project Officer may personally contact the subject.

So, in any study on occupational exposure, that is an aspect which has considerable importance. We have divided the groups to be traced into those estimated to be easy to trace, or of moderate or considerable difficulty. One of the things involved is to know when to stop tracing efforts because of no further leads. But we give free rein to those tracers in whom we have confidence, to determine leads if at all possible. They also tell us when they think that certain tracing efforts should be discontinued, because too much money has already been expended on particular subjects. We try to achieve at least 90 percent follow-up.

DR. SPIRTAS: I hear from Ken discussion of multiple causes and from George an interest in tracing, from Diane an interest in certain dusts.

MR. FOUNTOS: I was concerned about studies having adequate power. Some studies lack sufficient power to detect lung cancer, nasal cancer, laryngeal cancer, or other respiratory diseases among nickel-exposed workers when the risk of death from these sites is one and a half times that of the comparison population.

DR. AUSTIN: I am Don Austin from California. There are several methodologic issues that I am interested in. Of particular interest is one which points out a lack of legislation. We have been involved in a program in which we identified an excess of malignant melanoma in a rather large sized occupational group. We realized when we finally found it that it had begun about 10 years earlier. We were surprised that the medical facility at the plant hadn't found it because they had a very alert system. They brought in employees sometimes as often as every three months and gave them medical exams. They also had a very good crew of industrial hygienists who made sure everything was done according to standards.

We finally realized that the reason they had not noticed that they had an excess of this disease was because they were not keeping track of the number of cases of disease and comparing it to what might be -- they were not doing an observed and expected.

When we tried to figure out how we could implement such a program in the plant it became apparent that every hospitalizable disease was kept track through a third party payer who used it to set the rates for the premiums and didn't use it for any other purpose. It was quite possible under contract for the third party payor to supply these kinds of counts, by individual, to the employer. The employer, however, would not implement this kind of a program because if they found a problem it might result in a law suit against them, even though they had not been negligent. Although they had complied with all of the federal and state regulations, still they were afraid to look for problems because if any found, they might be sued for it. This points out a problem which might be solved through legislation.

Anyway, that is one methodologic interest I have. It started and it is being spurred now by the fear in industry, of looking for health problems because when they do, and if any are found, they will probably be sued for it.

DR. MARLAND: I am Richard Marland from the Environmental Protection Agency where I direct the Office of Research Grants. My interest in this program lies in my serving on a committee to make decisions regarding those projects which the collaborative program will fund. So I have a fairly broad interest in all of this.

DR. SPIRTAS: Okay, let me just mention one point that I want to get into the record, and then I will give the floor back to Barry, because we are running a little short on time. I guess I come back a little bit closer to what Don Austin said in that this morning I was at the General Accounting Office and there is an interest on their part, spurred on by a House subcommittee, to try to open up certain data resources in the Social Security Administration and the Internal Revenue Service to researchers.

One of the questions they have is how far do they go? Do they open it up merely to what I call protected government agencies, such as Census Bureau, which have very tight regulations, or do they open it up beyond that to research agencies such as NIOSH and the National Cancer Institute? Do they broaden that to EPA and OSHA which have regulatory functions, as well as research functions? Or do they go beyond that and open it up to universities which may not have as tight a control on the release of information as government agencies? How far do they go on this dissemination of information?

So there are people wrestling with this issue. I think in terms of methodology in an administration that is trying to find ways of cutting costs, we can certainly cut our tracing costs; we can certainly do studies of workers in the potash industry if that industry has a standard industrial classification code; we can certainly influence some legislation to have certain records, or to identify certain companies, if we could have access to the records of the Internal Revenue Service and the Social Security Administration.

To date we have had partial successes in some of these areas, and NIOSH has probably had the best track record in terms of some of the legislative efforts that it has made. So I wanted to get that point on the record.

We have about three more minutes. Barry, would you like to resume where we cut you off?

MR. FOUNTOS: Ken Bridbord had a question as to the recent evidence of prostatic cancer in workers exposed to cadmium. Although most of my time at OSHA has been spent researching the adverse health effects of workers exposed to nickel, and I have also set up a preliminary organization of the literature on cadmium, from an extensive literature search, by adverse effects on humans. And I was going to suggest that there are people at OSHA who have been working on cadmium for sometime and would probably be able to address any recent evidence of prostatic cancer among cadmium-exposed workers much more effectively than I could.

DR. SPIRTAS: Thank you. We have time for two more comments. Would anybody else like to get any other points on the record, or ask any questions or raise any issues? If not, then we can adjourn this session. I have about one minute to go. Yes, Ken?

DR. BRIDBORD: Almost seven years ago there was a fairly intense effort to bring together the current state of the art and knowledge on at least occupationally related cancer at the New York Academy of Sciences. I am just wondering if a similar effort might not be worthwhile in the emerging evidence from the laboratory studies, as well as emerging evidence from epidemiology.

It might take a week to bring this together, perhaps involving parallel sections. It would take at least a year to plan that type of a meeting and give people enough advance notice to pull together their information. But that might be a worthwhile activity. If planning started in the next three months we are probably talking about spring of '83 to have something like that.

DR. SPIRTAS: Let me just make two comments on that. In the spring of 1982 in the south of England, Surrey I believe, Dr. Acheson is sponsoring a seminar on exposure history dictionaries on how we create cross classifications of job titles with exposures.

Secondly, I believe Dr. Saffiotti has made a bit of an effort toward putting together a meeting under our NIOSH/NCI agreement.

DR. BRIDBORD: Particularly the laboratories.

DR. SPIRTAS: But I believe it has been broadened now to include the laboratory and the epidemiology groups. I believe that he is in the planning stages of this type of an effort to have a joint epidemiology-laboratory methodology session.

I take it what you are getting at is something that would have papers presented which would be published as proceedings?

DR. BRIDBORD: Yes. I think Umberto may have been approaching it principally from the point of the methodology. And I think that is a very important aspect of both. But also what I was talking about was what have people found in the last seven or eight years. If this would take place in '83, about the current state of knowledge in terms of completed studies, I think it might end up being a two-week effort of conferences, one dealing with methodology and the other dealing with what have we learned, so to speak.

DR. SPIRTAS: Any other comments? This session is now adjourned. Thank you.

PROCEEDINGS OF THE
SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP:
PROGRESS ON JOINT ENVIRONMENTAL AND
OCCUPATIONAL CANCER STUDIES

Friday Afternoon, September 11

DISCUSSION GROUP

GROUP B - EXPERIMENTAL METHODOLOGY/MODELS

Discussion Leader:
Dr. William Farland
Environmental Protection Agency

DR. FARLAND: This is our last session of concurrent discussions. We will just remind you once again, for those of you who are new with us, that what we are trying to do is to take a critical look at some of the projects that we have going under this collaborative program, and also to talk about some of the future directions in the program, and the types of work that we might like to see started under the auspices of this collaborative program.

Just as a review, we are at the end of the sessions, and a lot of comments have been made. We're at the point where we have talked quite a bit, now, about the fact that we are interested in seeing additional metabolism work in conjunction with short-term in vivo and in vitro testing, and development of biological markers, use of cell cultures and organ culture systems, in an effort to analyze some of the biological responses that we might be seeing. This work might allow us to get some handles on some of the biological responses that perhaps we are seeing when we are administering chemicals, or when we are looking at biological responses that have been caused by complex mixtures of chemicals, or environmental exposures.

We have had a good chance since the last discussion session to hear some additional things that are relevant to this topic. We have heard about histo-chemical and biochemical correlates of carcinogenesis, some protein markers in humans, use of hybridomas and monoclonal antibodies, 10T-1/2 systems and other transformation systems, use of some specific animal models in carcinogenicity studies, and use of wildlife populations as surrogates or bio-indicators or sentinels.

This is the type of thing, I think, that is the heart of this collaborative program, and we hope that we will be able to, in this session, have some critical comment, and some additional discussion on future directions of the programs.

I'd like to open up the discussion. I think that we are in a position at the EPA to be very concerned with a number of the topics that have been brought up this morning, in particular, the topic that was discussed earlier today on the issue of testing pure compounds or mixtures.

That topic relates, first of all, to animal testing for carcinogenicity, but it also relates to the interpretation of the results from short-term tests, and how one can use short-term tests in evaluating complex mixtures.

And I would like to stimulate a little bit of discussion on that point. Are there any comments, or any additional points that might be made, to start us off.

DR. NESNOW: I'd like to make some comments. I'm Stephen Nesnow with the Environmental Protection Agency. We have done quite a bit of work using the application of short-term tests to analyze complex mixtures. We have used both in vivo and in vitro cohort-term tests. One of our major programs is the use of the mouse skin tumor initiation, promotion, and complete carcinogenesis bioassays developed by Boutwell and co-workers. We have been using the sensitive SENCAR mouse quite extensively. This is in collaboration with Thomas Slaga at Oak Ridge.

We have looked at a large number of complex mixtures, including roofing tar emissions, coke oven emissions, and diesel and gasoline emissions, and have, of course, found a range of tumorigenic activities. Within a particular type of engineering class, like diesel, extracts of the diesel particulates vary from being totally inactive, to being highly tumorigenic, when applied dermally to these mice.

These studies have also been followed by fractionation of the complex mixtures into various components, and the bioassay of fractions; by in vitro bioassays, such as the Ames test, also the chemistry that goes along with identifying what agent or agents are involved in these mixtures is being investigated.

These studies are immense, because we're not dealing with a mixture of two or three agents. We're dealing with mixtures of thousands. Thousands of agents, comprising a wide variety of chemical classes. It is really very hard to put together a synthetic surrogate mixture that would mimic these effects from these complex mixtures.

I think one of the ways we have to go about this is to look at the complete mixture itself, and then to look at the various fractions, and then try to identify materials. This is a potential approach and one that has been fruitful in our laboratories.

I would also like to stress the need for futher development of short-term in vivo assays. The cost of doing a standard NCI bioassay is almost prohibitive these days, considering everyone's budget is going downhill rapidly, and in order to get this type of information, we need other types of assays. I'm referring to new bioassays developed which either use highly sensitive mice, or use a particular endpoint which develops very rapidly in their lifespan.

DR. FORD: Ford from the International Flavors and Fragrances. I would like to follow on Dr. Nesnow's comments with the observation I have out of today's presentations: complementing such bioassay screening studies done by NCI, is the continued development, or nurturing, of those studies that are going on, as, perhaps, with the hybridoma-antibody-classification of leukemic types. That technique and similar biochemical marker techniques can be used to classify and follow human disease and can then be associated with particular exposures and that can then be correlated with the screening information that came out of, for instance, mouse and rat skin painting studies.

I believe that that is where I would like to see the Federal programs move: to put into perspective some of the basic research that is being done. (and which should continue) and get those results off into something that can reflect back to health -- occupational health.

That concept leads almost to something we were talking about yesterday: collaborative programs between industry and Federal agencies.

DR. FARLAND: Yes, I think the point is well taken, both about the need for biochemical markers, in terms of understanding or detecting human disease, and the need to, let's say, putting in the exposure component with the biochemical markers for the detection of sites, or situations, that need to be investigated. As much collaboration as we can possibly have, to work out those types of problems, is going to be useful to us.

DR. CAMERON: Dr. Ford, I want to get back to our conversation yesterday afternoon.

DR. FORD: It would be really useful, yes -- really useful, but the critical point is there is no such real program of collaboration going on now. Maybe the first step would be this nurturing of the basic research into the epidemiological aspects.

The hybridoma antibody was being used only for classification as to which was the best therapy to use, but it has other applications. It can be used to associate a particular sort of leukemia with, possibly, a previous exposure, and that's where you get into the occupational health area --

DR. CAMERON: Dan, yesterday you mentioned to me the fact that you were in an industry that had some bad experiences in previous collaborative efforts with the government. Would you care to elaborate on that, just for a little bit?

Have we been making mistakes that have been nullifying our good intentions?

DR. FORD: I won't cite specific examples. When there has been a volunteering of information for either registration for compliance or with a survey, there have been just a few examples of a company's having demonstrated toxicity that has come back to haunt that particular company, when the information was sent by a jealous junior scientist to another agency or to the press.

To have one's test data turned around to bite you is what would discourage continued collaboration. If we could develop a program, such as your program of screening chemicals for anti-cancer activity -- once chemical companies realized that they could submit half a dozen to 3,000 different isomers of a chemical class, and have them screened for potential anti-cancer activity, and not have it turned around to bite them, I think you've probably got quite good cooperation.

By the business of "turning around to bite them," I mean for instance to have it released, to the press, that such-and-such chemical company is in the habit of producing chemicals that are anti-cancer agents. And, as you know, many of our anti-cancer agents are also carcinogens.

It's non-science, but it carries a lot of weight with your stockholders.

DR. SAFFIOTTI: I would like to follow up on some of the previous comments. This interesting discussion is trying to correlate the advances in research, the needs for developing test systems, and the use that we make of all this information to correlate it with human disease.

In looking back at the development of the field environmental and occupational cancer studies, we can see periods of activity in which the emphasis is on developing and defining new research methods, particularly methods that can be used for identification of etiologic agents, especially bioassay methods. In other periods the emphasis falls on studies of the underlying mechanisms.

For a number of years, we worked on the development and definition of animal model systems for carcinogenesis bioassay and related pathogenesis studies. In the last decade, the emphasis has been on the short-term methods, particularly the cellular methods and the indirect methods, such as mutagenesis, and DNA repair. I think the emphasis is now moving toward a more complex, but very exciting, period, in which we try to see how the different biological systems work and how they relate to human disease, which is the ultimate point of reference for us.

Some of these lines of work were developed in the laboratory. For example, my colleague Dr. Curtis Harris and co-workers have developed models for the culture of human target epithelial tissues such as bronchus, esophagus, colon, and a number of others. My colleague Dr. Stuart Yuspa with his co-workers have developed methods for the culture and transformation of epidermal cells, including methods selected to respond to tumor promoting agents.

Out of these lines of work we are just beginning to see the establishment of very promising cell culture models. For studies on the effects of chemical carcinogens on target epithelial cells, including human cells. I hope in the next decade we will be able to have a whole battery of target cell systems, including the human counterparts, to use for studies of human environmental and occupational carcinogenesis.

Now, I've mentioned the word battery, and that brings me to another point: I think that the emphasis is now moving more and more towards the need for well-designed batteries of biological systems for studies of toxicology, including carcinogenesis, because any single system can really be very difficult to interpret precisely in relation to human risk.

Some very interesting data, for example, were presented earlier today, by Drs. A. Sivak and L. Schechtman showing that the 10T-1/2 cell system did not respond to some classical carcinogens like aromatic amines but became capable of doing so under special conditions which were developed to overcome the lack of sensitivity.

We have been working the past two years with the BALB/c 3T3 clone A31-1-1 system developed by Kakunaga and we have found it to be particularly susceptible to transformation by a broad spectrum of carcinogens. We have had good transformation, not only with polycyclic hydrocarbons and MNNG, but also with aflatoxin and with benzidine. But I presume that, as we study that system more, we will find some categories of agents for which it will be not as good as for the others.

What seems to be needed is to work towards a combination of testing and research in this area, with the goal of developing batteries of test systems of known characteristics.

For many years now we have heard discussions about batteries of test systems. They seem to be taking a more effective place in our testing programs than the earlier proposals of tier systems, in which one system was good enough to exclude the others, and to be used as a yes-or-no answer before one went to any further step.

We need to rely on batteries of systems in this respect. What concerns me is that we should now try to develop the best mix of innovative research and applied studies, including testing.

As some of you know, I have personally been very concerned about the organizational separation of testing responsibilities from research responsibilities in the field of carcinogenesis in the programs of the Federal government, that followed the initial separation of bioassay from research in the NCI. This separation sense, has continued with the establishment of the NTP as a separate entity.

But now I see all of us, from different agencies and programs, trying to get together under the same roof, and to orientate our research and our testing efforts so that they interdigitate as much as possible.

Industry, I think, can help in this direction, by doing more than testing its own products, or fighting for legal criteria of classification and regulatory matters: industry could be contributing substantially to the support of research that is directed to providing the understanding of the critical chemical-biological interactions. Some individual companies or industry groups have already given, historically, a very important contribution to research. Some of the drug industries and their research institutes, for example, have contributed substantially and very effectively to basic research in some of these areas. But I have a feeling that a lot more could be done. With the prospect of the Federal budget being further restricted in many areas, including this one, there will be a need to pool our strengths together somehow. Research in this field should not be just left floating around in a totally serendipitous way, but should be focusing on areas that can be connected with the practical problems of human health. The testing programs, on the other hand, should be considering specifically how much of their total effort ought to be devoted to the elucidation of some of the basic mechanisms that are the key to our evaluation of biological responses.

DR. FARLAND: Thank you, Dr. Saffiotti. I think, if I might just make a comment -- and either yourself or someone else respond to it -- that is relevant to some of the points that you are making. One of the hard questions that faces us, if we start to take this type of an attitude -- and I certainly don't know the answer, and I would like to hear some discussion on it -- are we to, at this point, say that we are interested in the direct correlates with human disease? We would like to have some markers for those diseases, we would like to have some short-term tests, with human cells and tissues, that might correlate directly, and be used experimentally to make some determinations that might allow us to look at hazard associated with certain types of chemicals. But, are we willing to make statements directly from the responses of these tests to hazard to humans?

Or are we, as we have done in the past, thinking about tests which will eventually lead us to that old statement: Well, this is the one we have to test in an animal?

DR. SAFFIOTTI: I have just come back from a NATO course on "The Use of Human Cells in the Assessment of Risk From Chemical and Physical Agents" which reviewed recent advances in cell transformation, including transformation of human cells, and genetic toxicology studies related to human cells. The information question for us is how can we use all this biological information, estimate hazards, and assess risks for the human? The consensus mostly was that qualitative risk assessment is still a much more reasonable goal than the attempt to make quantitative risk assessments.

But then, qualitative risk assessment has not been pursued in a very systematic way, except for general statements that if a given agent is carcinogenic in test animals it's likely to be carcinogenic in other species, including the human. There is a lot more to a qualitative comparison than this. One can really compare the metabolic pathways, the susceptibility states, and many parameters that define the biological response in the target tissues. A lot of this work is beginning to become feasible.

The time may have come to try and focus on some selected examples, take groups of substances as test cases, and study them in a variety of biological and biochemical model systems, to see how they correlate within systems and with the human.

DR. FARLAND: Any additional comments?

DR. CAMERON: Getting back to the industrial-government cooperation, I can think of two things. One that we all know about now is the NCI-Formaldehyde Institute collaborative study, and I think that's a prime one. As I mentioned yesterday, I was very thankful that they came to us.

The other striking example, I think, right now, is that we have been working for well over a year with DETO -- that's the Dye Manufacturers' Association of the United States, on a class study of the dyes.

Now, again, that was a mutually beneficial undertaking -- their thrust is that they can't possibly pay for an in-depth study and testing of over 2,000 to 3,000 dyes that they make, or manufacture, or import, and they're coming to the NCI and trying to find out, in this class study, can we select a spectrum from the various classes, and subject those to intensive, in-depth study, and by that means extrapolate to the whole field of dyes?

I think it's a great idea. I don't know if it will work, but if it would work with dyes, I think we could move on the other areas.

DR. GREGORY: If it will work with dyes, it will work with anything.

DR. CAMERON: Yes, Bud's been involved. He'll appreciate the problems with it.

Dr. Gregory, that goes back to what Dan said, too, though. We have to have the test systems well validated and well established before we can hope to expand them to that use.

DR. WEISBURGER: Weisburger. I'd just like to mention that another example of this government-industry-academia collaboration is the thiolate -- the study which came out of the workshop that was held back in June, where apparently the CIIT is going to do lots of projects, and it will all be coordinated through a sort of clearinghouse, and I think that's an excellent example of cooperation.

DR. FARLAND: I think the application of as much knowledge as we can gain on these, from all sources, is going to allow us as scientists to make some rational decisions on these chemicals, and that, once we get past the idea that any one test is going to give us the answer that we need to determine human risk, we really have taken that big step, and we're at a point where, as scientists, we really can make some good judgments.

DR. LONGFELLOW: Longfellow, NCI. This comment probably would be more appropriate over in Section A, but while we're mentioning the cooperation aspect, it seems to me that, if you listened to the story of the epidemiologists over and over again, the last day or so, the picture is one of always having to reinvent the wheel.

That is, you walk into a facility, and you say, oh, my goodness, the records aren't structured so that I can get what information I need, and if we only had this, we could include the 26 percent of the employee population that John found missing --.

If, in fact, we were to look at the record system, in terms of what is really needed for the ideal epidemiological study, and get the sort of cooperation right at the beginning since we're going to need these years down the road, and we're going to find new settings where new chemicals are being made.

If you think of it, the one thing that we can change and have some control on now, which is to get employee and patient records structured so that one could walk in and do a much more complete and comprehensive kind of study. I think that's something that this sort of interagency interaction could well afford to put some energy into. Some attempt to develop a model record keeping system with this end in mind should be developed.

DR. NESNOW: Send it out as a recommendation to everyone -- academia, government, and industry.

DR. FARLAND: If there are no further comments, we'll close up this session. If there are any additional comments that you would like to make, after you think, and want to see me before our final summary session, I would be glad to listen to them.

Thank you.

PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

Effect of Varying Doses of UVB and UVA on Mammalian Skin

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EFFECT OF VARYING DOSES OF UVB AND UVA ON MAMMALIAN SKIN

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Decatur, Georgia.

The effect of UV radiation on skin is of interest to us from both a scientific and a medical standpoint. As scientists who are directly involved with clinic aspects of cutaneous photobiology, we are acutely aware of the potential deleterious effects of excessive exposure to sunlight and of the fact that on-going and predicted ozone depletion is likely to increase, not lessen, the risk of such effects. The major malignancies and premalignancies unequivocally associated with sun-exposure include solar keratoses, basal cell epitheliomas, squamous cell carcinomas and keratacanthomas.

Squamous cell carcinomas are the most prevalent form of all cancers, and they have the potential being metastatic. It is estimated that there are 300,000-500,000 cases of non-melanoma skin cancer per year in the U.S., which represents 1/3 to 1/2 of all cancers (1). Of these, approximately 50,000 die and a large percentage are severely mutilated (i.e., loss of nose, eye, or major portions of other areas). Since the morbidity and costs are extremely high, there is a severe effect on the economy. Billions of dollars are being spent for treatment, and the drug industry has committed a major effort to the development of sunscreens. Malignant melanoma has also been linked to sunlight exposure, at least epidemiologically. It has been observed that the incidence of melanoma increases during or shortly after periods of high sunspot activity.

The problem of ultraviolet carcinogenesis is complex. The environmental effects, which play a very significant role, involve geographic, temporal, anatomical, and life-style-related considerations. Perhaps the most important of these is the modification of the stratospheric ozone layer by human activities. No studies which seek to predict ozone effects on carcinogenesis can be carried out without a basic knowledge of how alterations in solar spectral distribution and intensity which would occur on ozone depletion would influence UV photocarcinogenesis. Among the factors which must be considered are (a) relative effectiveness of different wavelengths in photocarcinogenesis, (b) dose-response relationships, (c) time-dose effects, (d) repair processes (e) immunologic considerations and (f) effects of chemical co-carcinogens.

Although it is generally agreed that the UVB portion of the solar spectrum (i.e. 290-320 nm) is most effective in causing skin cancer, knowledge of the relative effectiveness of these wavelengths is not well known (i.e., reliable action spectra do not exist). Moreover, the UVA region (320-400 nm), which was long thought to be unimportant in UV carcinogenesis, is now known to augment UVB sunburn response. The evidence is now clear that UVA can also augment UVB carcinogenesis, even though a poor carcinogen in its own right (3). Because of the above-mentioned complexities, time-dose response characteristics are not known to the degree to which is required for the explanation, prediction, and subsequent prophylaxis of carcinogenic effects.

In recent years, concerns have been raised that environmental chemical and physical events may adversely affect skin cancer incidence by affecting the stratospheric ozone shield. The ozone shield removes more than 99% of ultraviolet radiation of wavelengths less than 320 nm from the incoming sun's rays (2). Man could reduce the stratospheric ozone concentration by a variety of ways including thermonuclear explosions, exhaust gases from space rockets, SST's, etc. and halomethanes used as spray propellants.

The overall aim of this work is to undertake a systematic study of the dependence of squamous cell cancer development on wavelength distribution (broad and narrow band), total dose, and dose fractionation in a manner simulating solar radiation at various stages of ozone depletion. Our unique inbred strain of the Sk-1 hairless mouse serves as a good animal model. These mice have been inbred in our laboratories for at least 30 generations and are available in large numbers at all time. Our experiments clearly show that SSR can reproduce these cancers in a consistent predictable manner in these mice.

METHODS

To better understand the inter-relationships between solar carcinogenesis and alterations that might occur as a result of ozone depletion, we embarked upon investigations to determine the rate of squamous cell cancer induction in 6-week old Sk-1 hairless mice by chronic exposure to SSR (solar-simulating radiation) from a 1.6 KW Xenon arc lamp passed through Schott WG-320 filters of thicknesses varying from zero to 3 mm. These filters allow a simulation of various conditions of UV solar radiation that one might expect to occur under ideal environmental conditions of varying extents of stratospheric ozone depletion. The spectral emission for each filter combination is shown in Figure 1. The effect of using the various filters is to alter the UVB component while leaving the UVA essentially constant.

In other experiments in the effects of relatively narrow band UV radiation on development of squamous cell carcinoma in hairless mice were studied. A xenon-mercury arc in conjunction with a Bausch and Lomb grating monochromator (10 nm half-hand width) was set at 280, 290, 300, 307, 313, 366, and 406 nm.

Hairless mice were irradiated on the mid-dorsal surface on a 1.5 cm^2 square site. Minimal erythema dose (MED) determinations were made (Figure 2). The lower curve in Figure 2 is the MED for each combination as a function of Schott WG 320 filter thickness (top axis), and the upper curve is the reciprocal MED ("action"). The lower axis represents the wavelength where the light intensity is 1% of its value at 340 nm. Figure 2 shows that erythemal efficiency is markedly enhanced by short wavelength components.

Chronic Experiments: Sets of 20 Sk-1 hairless mice were irradiated with each filter combination according to our rapid tumor induction technique (3). In this regimen, the daily dose was increased by 20% increments of starting dose (D_o) every 6th day for 40 irradiation days. Clinical responses were graded on a 6 point scale (Table I). Histological responses were also monitored by routine H&E and PAS staining.

Two types of experiments were carried out: (1) Irradiation at equal dosage (D_o = 6.5 J/cm^2), (2) Irradiation equivalent to equal biological effect with D_o = 0.5 MED in one set of the experiments, and D_o = 0.9 MED in the second set. The endpoint for cancer development was taken as a 3+ reaction, which corresponded to early malignant development as defined by Epstein et al (6).

RESULTS AND DISCUSSION

I. Solar Simulator Experiments:

A. Dose equivalent Equal Effect: The next 5 figures show dose response curves for the various filter combinations. Figure 3 (no filter combination) shows trends which generally hold for the entire series:

a) Tumors are observed at lower dosages for the 0.5 MED group than for the 0.9 MED group. However, it should be pointed out that all groups took approximately the same time (15-18 days) for tumor production to manifest itself.

b) A higher percentage of tumors or papillomas were produced in the 0.5 MED group than in the 0.9 MED group, at least in the early stages. Many of these later regressed. Regression was present, but not obvious in the 0.9 MED group.

Figures 3-7, show dose response data in order of increasing filter thickness. From these figures, we notice also that: c) the shorter wavelength components appear to preferentially produce tumors, since the dose required to produce tumors increases with increasing filter thickness.

d) with increasing filter thickness, tumor production tends to become less efficient for both groups, but the efficiency of the 0.5 MED group is especially decreased.

B. Equal Dose Experiments: Irradiations were carried out for each filter combination at $D_c = 6.5\ J/cm^2$. These doses correspond to 0.9 MED for "no filter," 0.5 MED for 0.5 mm filter, and 0.3, 0.28 and 0.24 MED for 1.0, 2.0, and 3.0 mm filters respectively. The results of the first two experiments have been discussed above. Some precancerous response (1+) was obtained in 75% of the animals at 1.0 mm, but no tumors were noted. No response was obtained for 2.0 & 3.0 mm filter groups.

Several salient features arise from these results: Firstly, decreasing the ozone layer (thinner filters) will lower the dose required for tumorogenesis. Secondly, photocarcinogenesis is a dynamic process; tumor growth competes with "dark" regression. This can be seen from: (a) the behavior ("waxing and waning") of the 0.5 MED dose response curve (b) the lack of tumorogenesis in the 1,2 and 3 mm "equal dose" experiments, where low doses (D_o= 0.25 MED to 0.3 MED) were used. Thirdly, there is a distinct lack of correlation between erythema and tumor development. The 0.5 MED groups develop tumors at lower doses and higher efficiency than their 0.9 MED counterparts (at least in early stages).

II. Monochromator Experiments

Figure 8 shows the energy action spectrum for erythema production. This curve peaks at ca 300 nm, in agreement with results found by other workers. Quantum correction does not drastically change the shape of the spectrum.

Next, dose-response curves were constructed for the different wavelengths using the 0.5 MED and 0.9 MED regimens. Figures 9 and 10 show the responses for 1+, 2+, and 3+ reactions at 313 nm for the 0.5 MED regimen. The latter reactions later regress, even though the daily dose is incrementally increased. For the 0.9 MED case, there is much less tendency for regression. We eventually obtained 4+ reactions (not shown) with 0.9 MED, but not 0.5 MED regimen. These figures illustrate some points which seem to be characteristic of all wavelengths: (1)

for the 1+ response, the 0.5 MED appears to be more efficient than the 0.9 MED because lower total doses are required to elicit the response. On the other hand, the time required to produce the response is similar in both cases. This parallels the results of the solar-simulator experiments. (2) The 1+ response seems to track with erythema response. (3) Reciprocity is not obeyed, as is clearly seen in the 3+ response for the 0.5 MED experiment (Figure 9).

At lower wavelengths, there was significant difficulty in producing cancerous changes. The situation at 307 nm is illustrated in Figures 11 and 12. There is no attainment of 3+ response in the 0.5 MED case, and the 2+ response exhibits marked regression. In the 0.9 MED regimen, there is some (20%) 3+ production at early stages.

This trend is even more pronounced at shorter wavelengths. At 300 nm, the 2+ stage is obtained with difficulty (Figure 13, 14),and the dose-response is very complicated. The 2+ stage is eventually reached in the 0.9 MED group (not shown), but no higher response was attained. At 290 nm, only one out of five mice reached the 2+ stage after prolonged radiation for the 0.5 MED experiments,and two out of five reached 2+ for the 0.9 MED experiments (Figure 15-16). At 280 nm, only the 1+ stage is reached at all for both 0.5 and 0.9 MED (Figure 17-18).

These results can be re-expressed as action spectra: Figure 19 is the action spectrum for 1+ development for each regimen. All mice developed response on same day, so that each point represents the point where 100% of the mice showed responses. The shape is the same for both spectra and these are, in turn, the same as for erythema. The efficiency for the 0.5 MED group seems to be higher than for 0.9 MED group. Figure 20 represents a "best approximation" for the 2+ response. The numbers in parentheses represent the fraction of mice exhibiting the response. It was not possible to construct complete spectra for 3+ or 4+ responses, since no such responses were obtained in many cases.

The salient point of these experiments with "monochromatic" wavebands is that, on chronic irradiation, the efficiency of tumorogenesis seems to markedly decrease. Consequently, the apparent maximum "action" shifts from 300 nm for the 1+ response to 307 nm for the 2+ response. If the action spectra for more advanced responses could be obtained (which would require more stringent radiation conditions) the apparent shift of the peak action might well extend to longer wavelengths. These results may be due to several features: (1) increased stratum corneum/epidermal thickening. Such thickening leads to increased skin absorption in the 280 nm region (5) which results in a selective attenuation of 280-300 nm radiation, so that less light reaches the target cell. It affects the 300-313 nm less drastically. (2) Wave-length dependent differences in repair processes. For example, photochemical reversal of thymine dimers could occur at wavelengths less than 300 nm, but are unlikely above 300 nm. (3) Possible differences influence rate and (4) Possible UVA/UVB waveband interaction (i.e. photoaugmentation) at wavelengths close to the UVA region. The large half-value band width of the monochromator (a condition chosen to increase energy through-put) makes such interaction likely, especially at 313 nm.

SUMMARY AND CONCLUSIONS

Photocarcinogenesis is dynamic. There is a marked tendency for simultaneous opposing reactions, which gives rise to complicated dose response patterns. The apparent action spectrum shifts on going from early to later stages of tumorogenesis which makes the concept of "the" action spectrum questionable in this case. The observed shift may be result of stratum corneum thickening, waveband interactions, differences in repair processes and/or other phenomena. There is not a simple relationship between erythema and tumorogenic responses. Even at early stages where both action spectra are similar, the lower dose regimen more efficient at 1+ response production.

The Significance of this work is that it: (1) underscores the dynamic nature of tumorogenesis, (2) underscores non-simple relationships between spectral distribution (with possible waveband interactions), total dose, dose fractionation. (3) underscores the need for more detailed information which is necessary for assessing the effect of man-made ozone depletion. (4) can form basis of future work which will increase our mechanistic understanding of photocarcinogenesis and impact of ozone depletion thereon. (5) can provide insight into prevention of tumors.

Most urgent future work involves (1) a closer investigation of fluence rate (excitation intensity) on tumorogenesis and (2) the effect of irradiating large areas. The importance of the first study is implicit in the above discussion, whereas the second is clinically relevant to the human situation. Longer term goals include further molecular mechanistic studies (e.g. monitoring the effects of appropriate biochemical markers of UV-induced damage) as well as gauging the effects of such factors as immunologic surveillance and chemical co-carcinogens on UV photocarcinogenesis.

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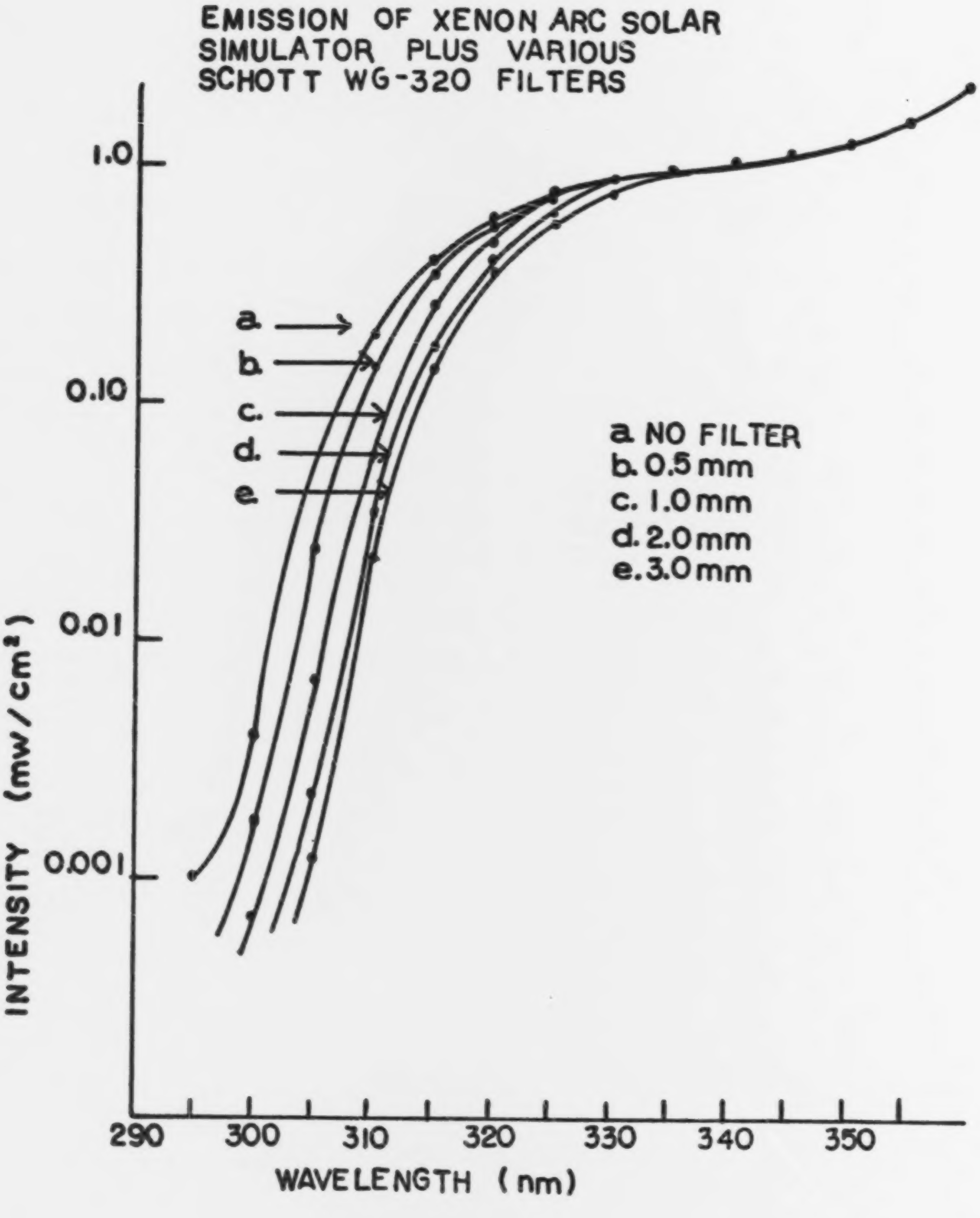
TABLE 1

Grading Scale of Erythemal and (Pre) cancerous Clinical Responses of SK-1 Hairless Mouse Skin to UV Radiation.

E-1	Mild to moderate macular erythema
E-2	Intense macular erythema
1+	Light scaling accompanying erythema
2+	Firm scaling, palpable keratosis
3+	Raised palpable, keratotic plaque corresponding to early malignant development as defined by Epstein et al (6)
4+	Papilloma or tumor corresponding to extensive malignant development

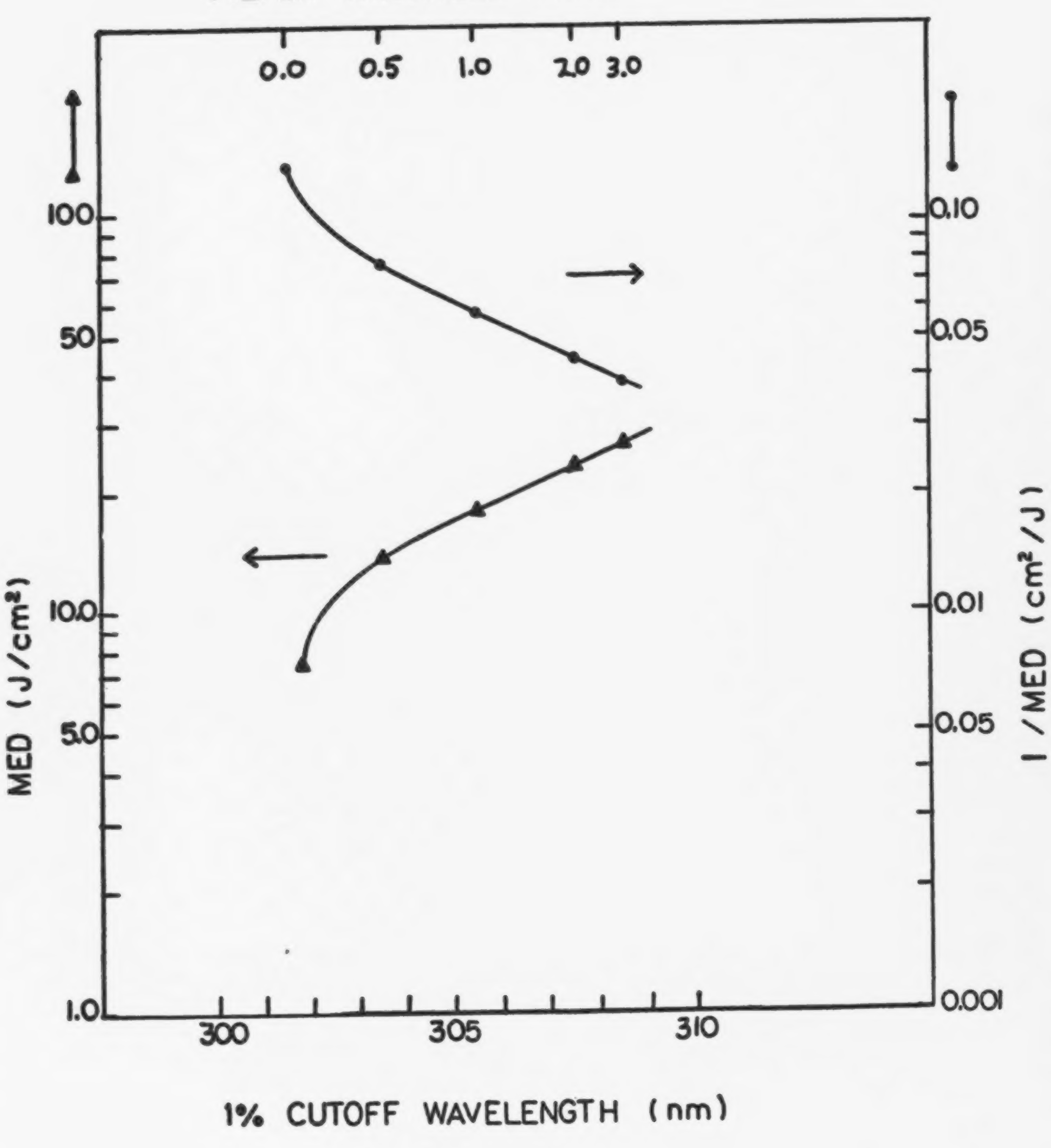
The E-1 and E-2 responses are not considered to be precancerous; they are simply sunburn responses. The 1+ through 4+ responses represent a continuum of precancerous and cancerous changes.

Figure 1



BEST DOCUMENT AVAILABLE

Figure 2



FILTER THICKNESS (mm)
0.0
0.5
1.0
2.0
3.0
100
50
10.0
5.0
1.0
MED (J/cm^2)
0.10
0.05
0.01
0.05
0.001
1 /MED (cm^2/J)
300
305
310
1% CUTOFF WAVELENGTH (nm)

DOSE - RESPONSE OF
IRRADIATION OF SK-I HAIRLESS MICE WITH
290 - 400 SOLAR - SIMULATING RADIATION
NO WG-320 FILTER

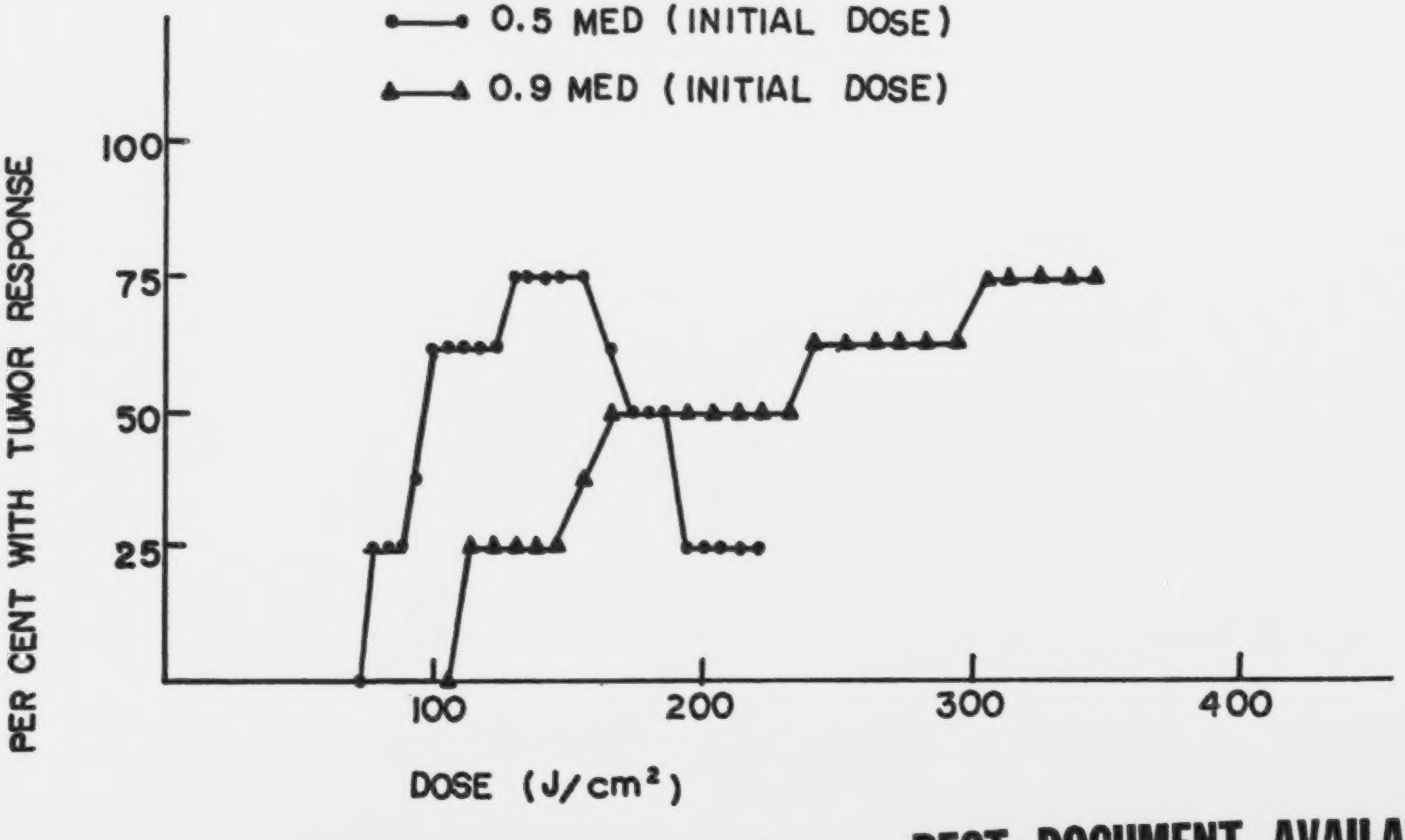


Figure 3

DOSE RESPONSE OF
IRRADIATION OF SK-1 HAIRLESS MICE WITH
290 400 nm SOLAR-SIMULATING RADIATION
WITH 0.5 mm WG 320 FILTER

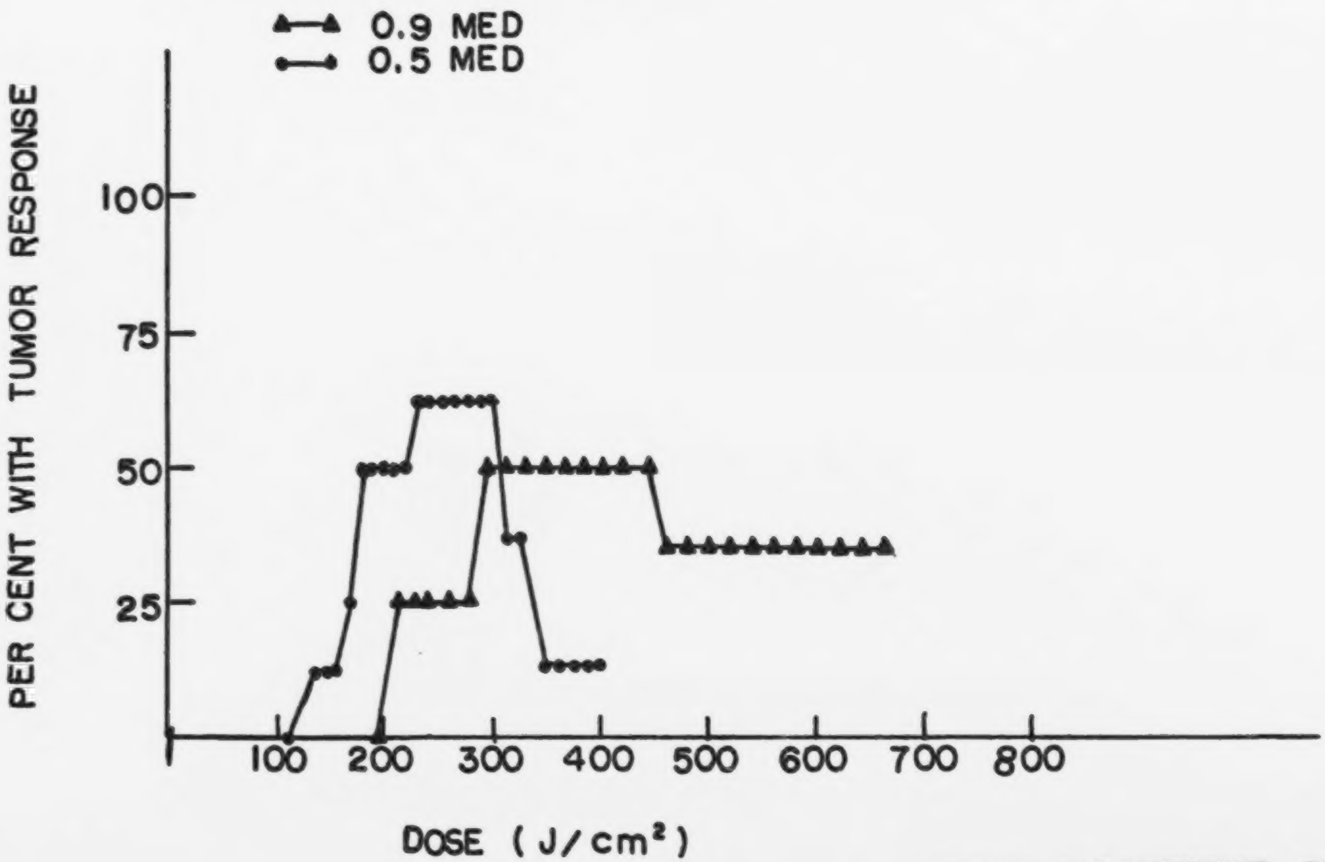
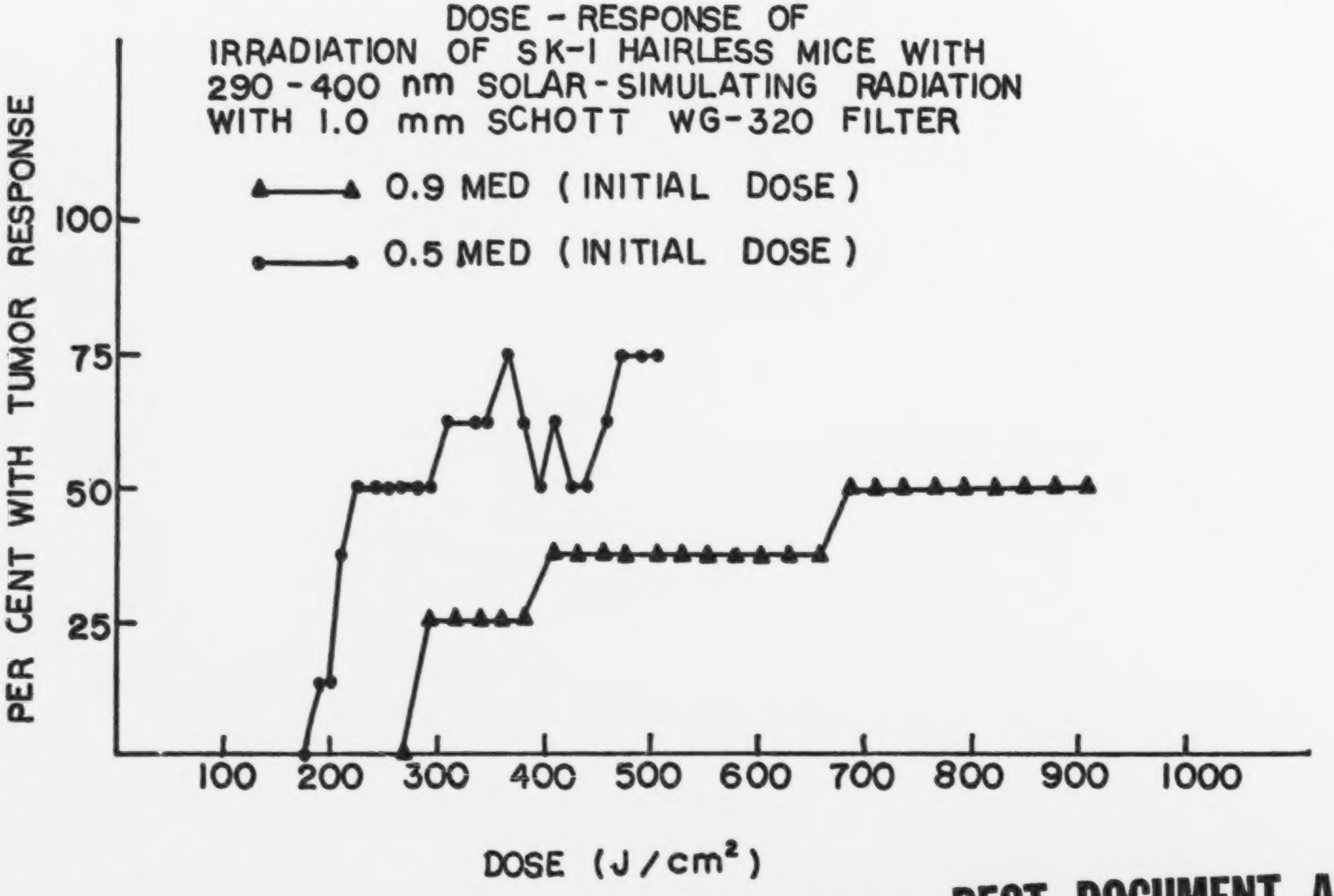


Figure 4

751



DOSE - RESPONSE OF
IRRADIATION OF SK-1 HAIRLESS MICE WITH
290 - 400 nm SOLAR - SIMULATING RADIATION
WITH 1.0 mm SCHOTT WG-320 FILTER

Figure 5

752

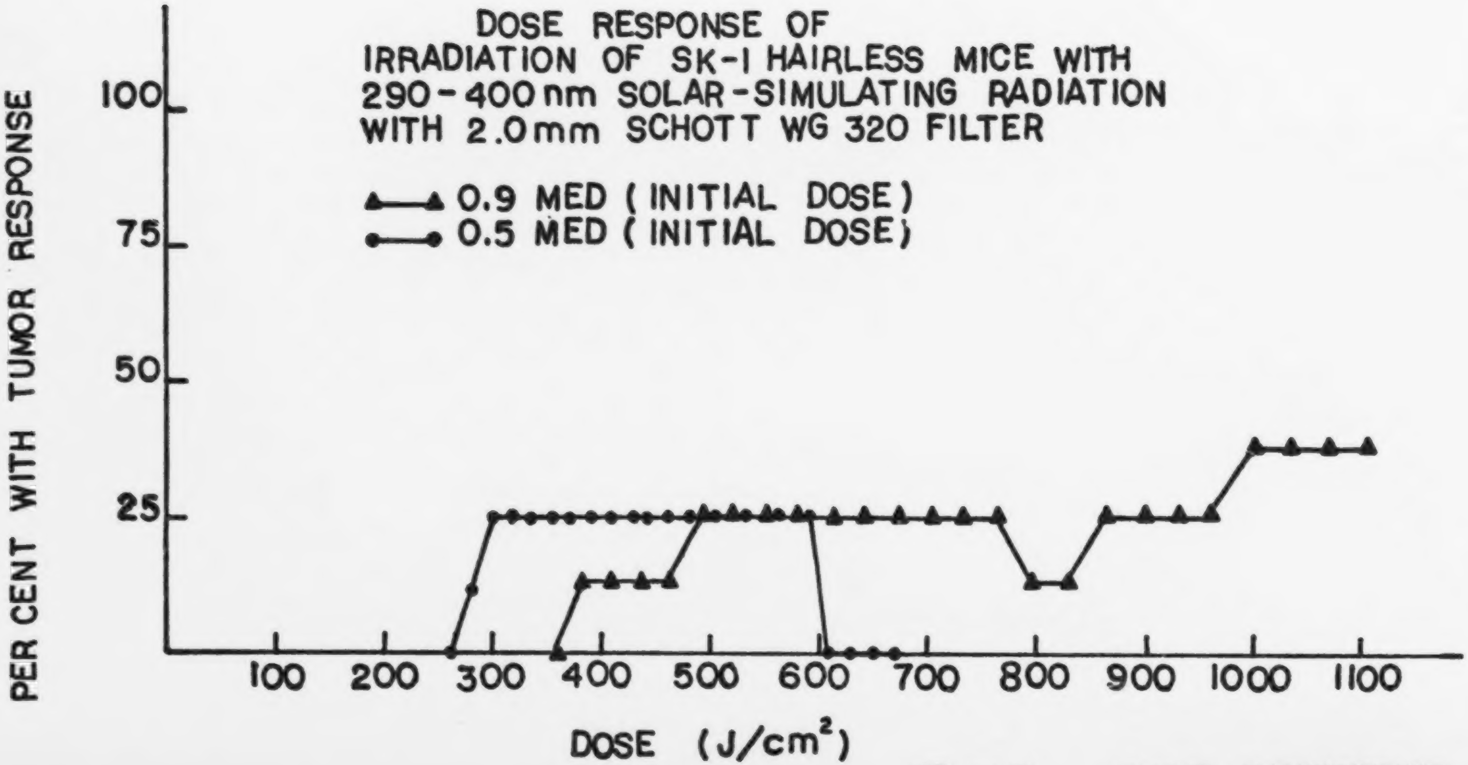


Figure 6

753

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DOSE RESPONSE OF
IRRADIATION OF SK-1 HAIRLESS MICE WITH
290-400 SOLAR-SIMULATING RADIATION
WITH 3.0mm SCHOTT WG 320 FILTER

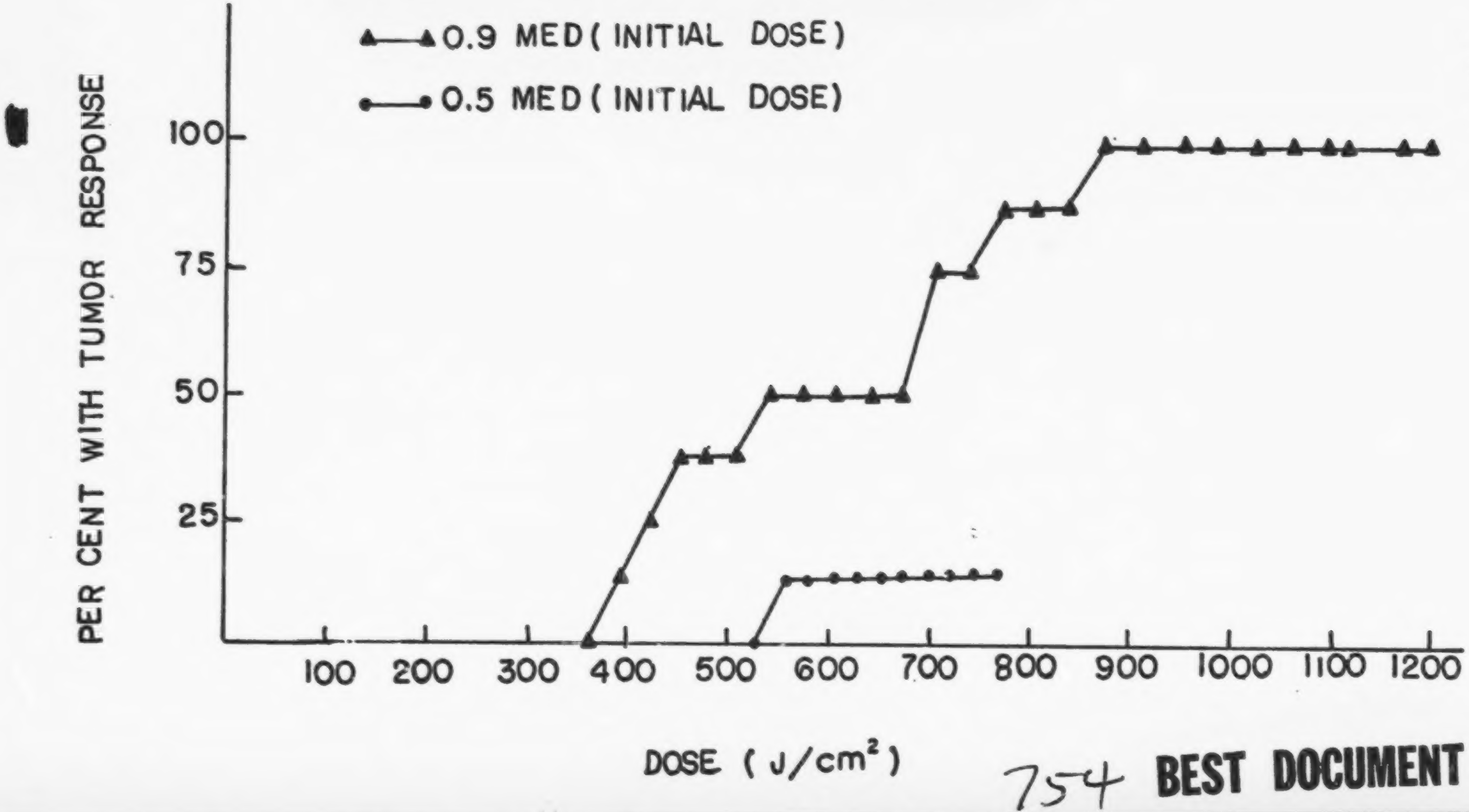
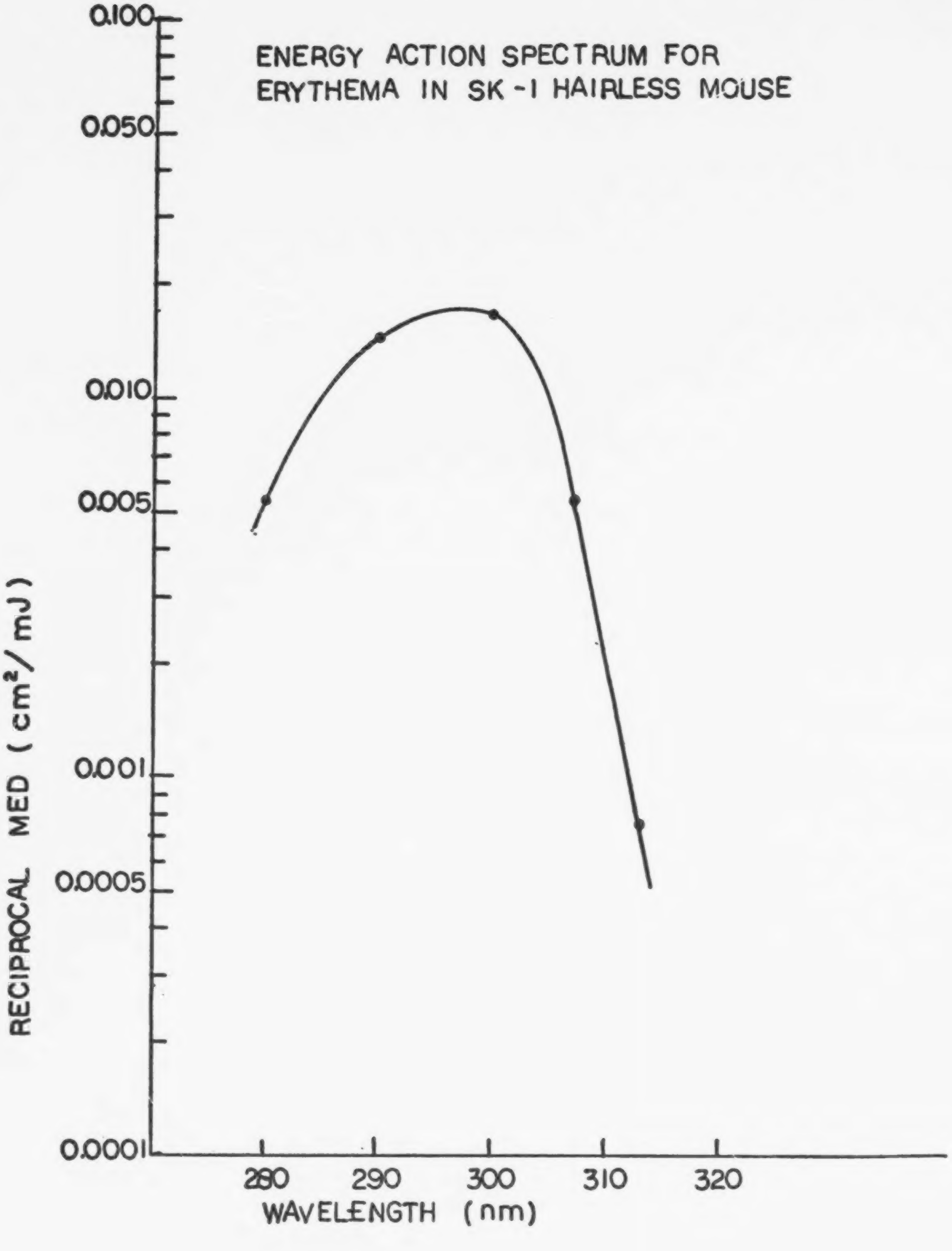


Figure 7

754

Figure 8


ENERGY ACTION SPECTRUM FOR
ERYTHEMA IN SK-1 HAIRLESS MOUSE
0.100
0.050
0.010
0.005
0.001
0.0005
0.0001
RECIPROCAL MED (cm^2/ mJ)
280
290
300
310
320
WAVELENGTH (nm)

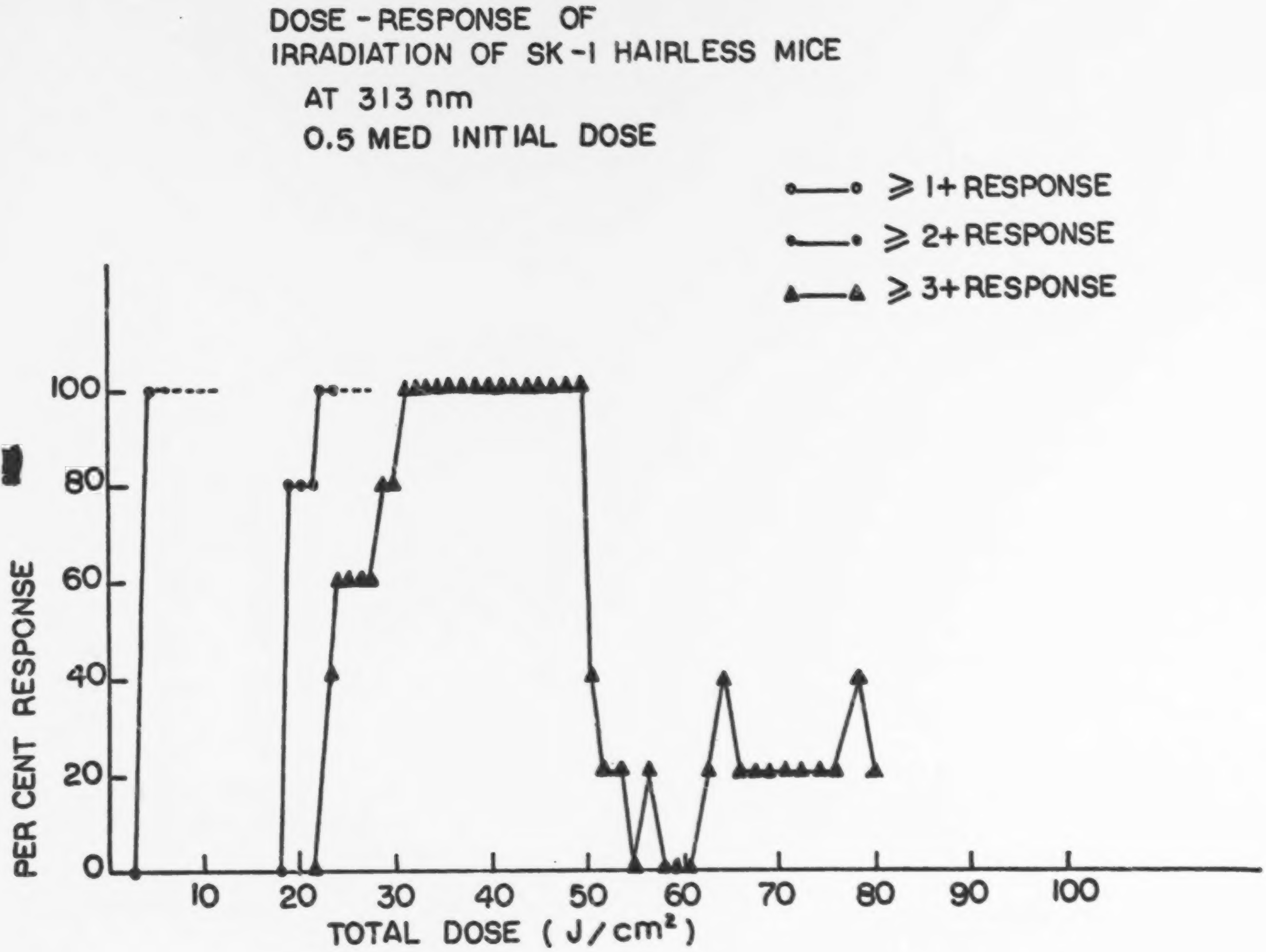

DOSE - RESPONSE OF
IRRADIATION OF SK-1 HAIRLESS MICE
AT 313 nm
0.5 MED INITIAL DOSE

Figure 9

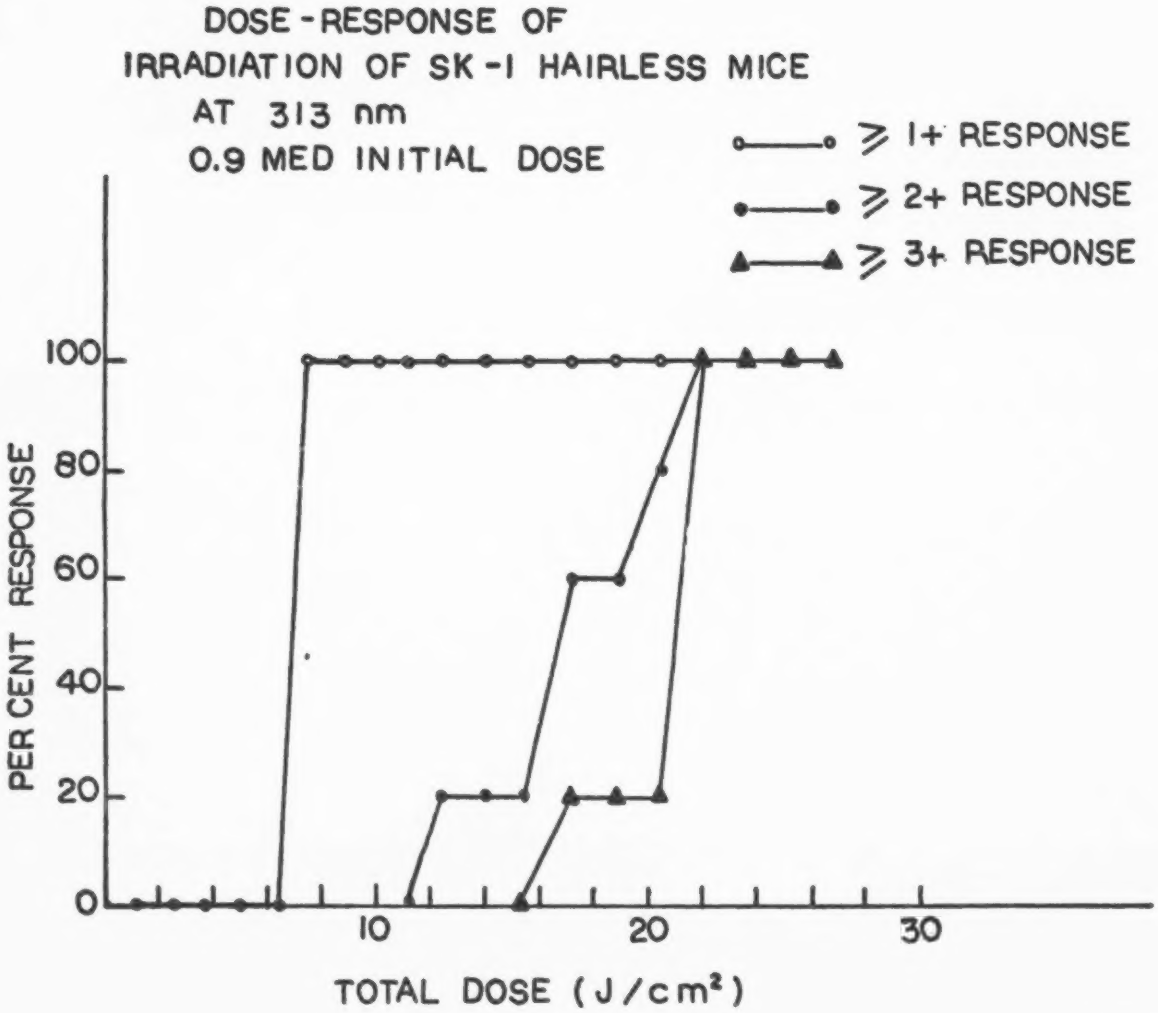
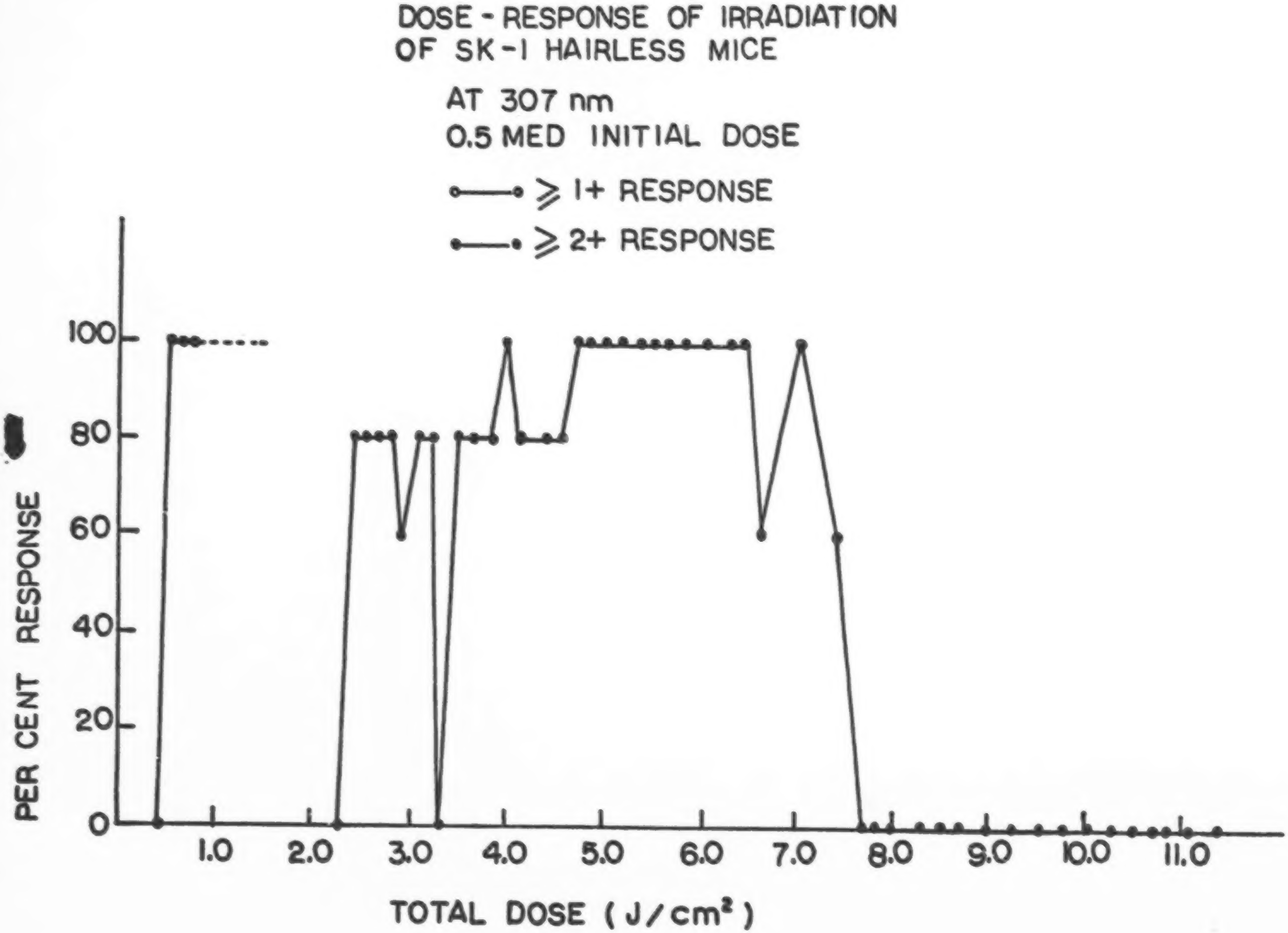

DOSE - RESPONSE OF
IRRADIATION OF SK-1 HAIRLESS MICE
AT 313 nm
0.9 MED INITIAL DOSE
≥ 1+ RESPONSE
≥ 2+ RESPONSE
≥ 3+ RESPONSE
PERCENT RESPONSE
100
80
60
40
20
0
10
20
30
TOTAL DOSE (J/cm²)

Figure 10



DOSE - RESPONSE OF IRRADIATION OF SK-1 HAIRLESS MICE
AT 307 nm
0.5 MED INITIAL DOSE

Figure 11

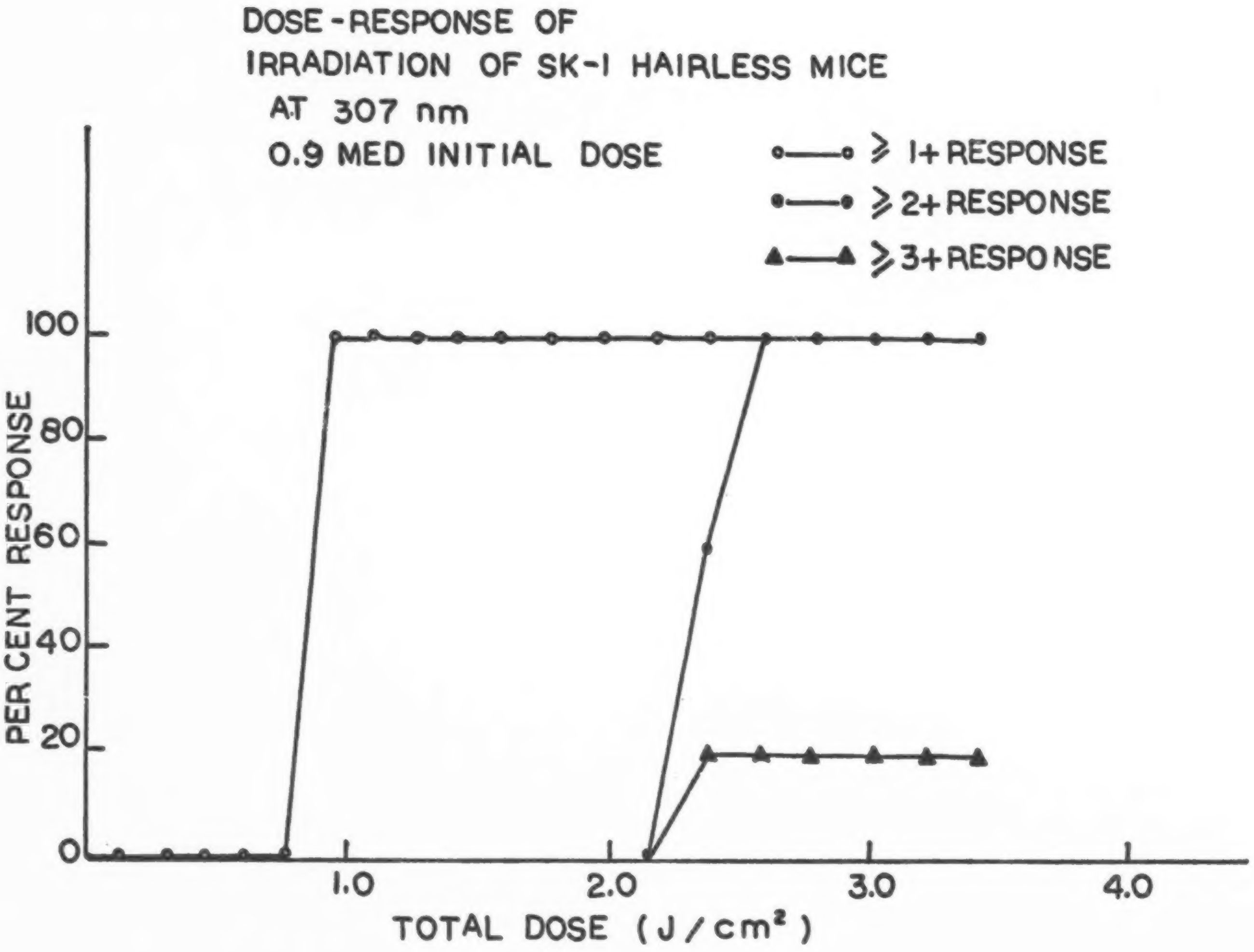

DOSE-RESPONSE OF
IRRADIATION OF SK-1 HAIRLESS MICE
AT 307 nm
0.9 MED INITIAL DOSE
≥ 1+ RESPONSE
≥ 2+ RESPONSE
≥ 3+ RESPONSE
PER CENT RESPONSE
100
80
60
40
20
0
1.0
2.0
3.0
4.0
TOTAL DOSE (J/cm²)

Figure 12

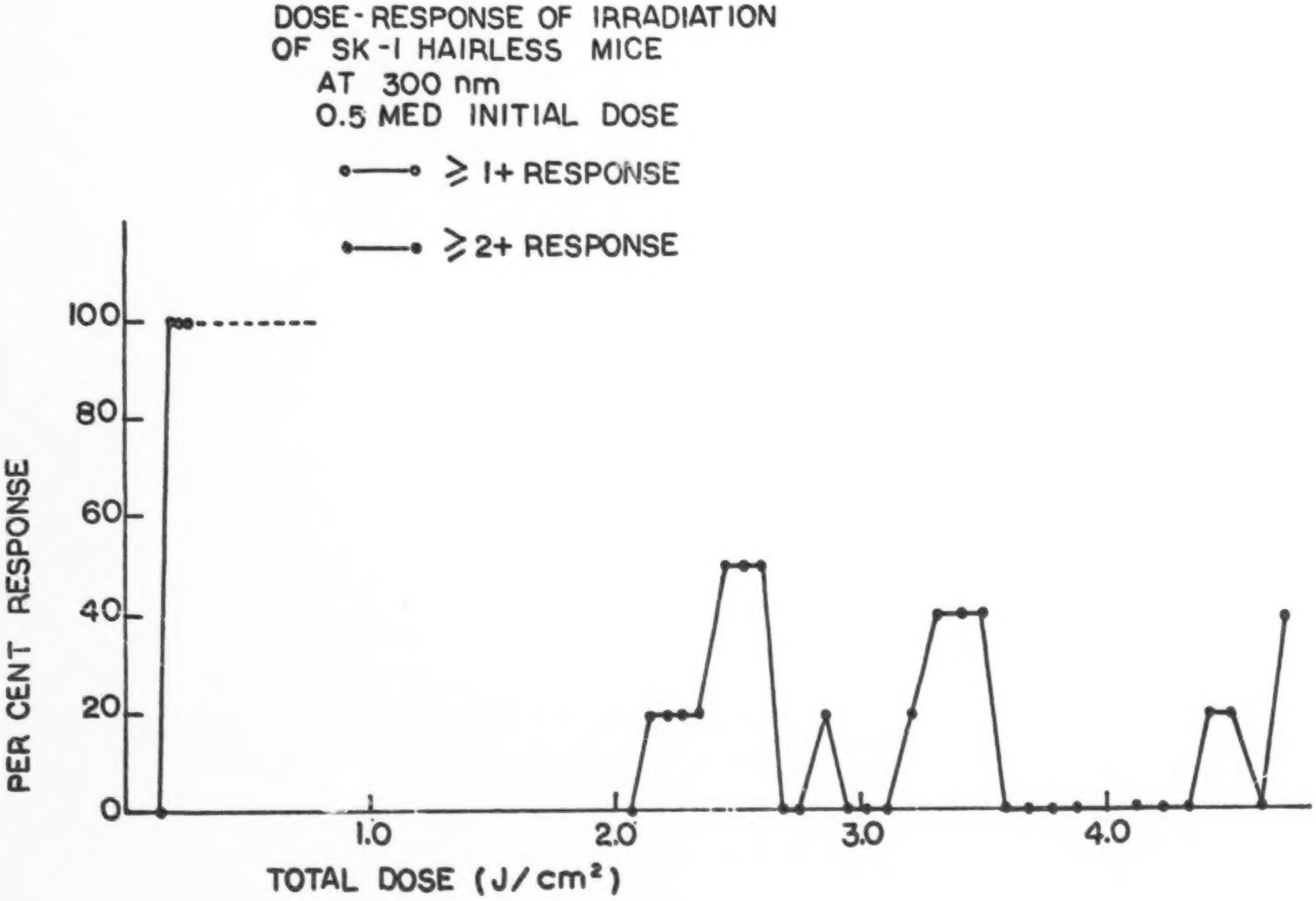


Figure 13

760

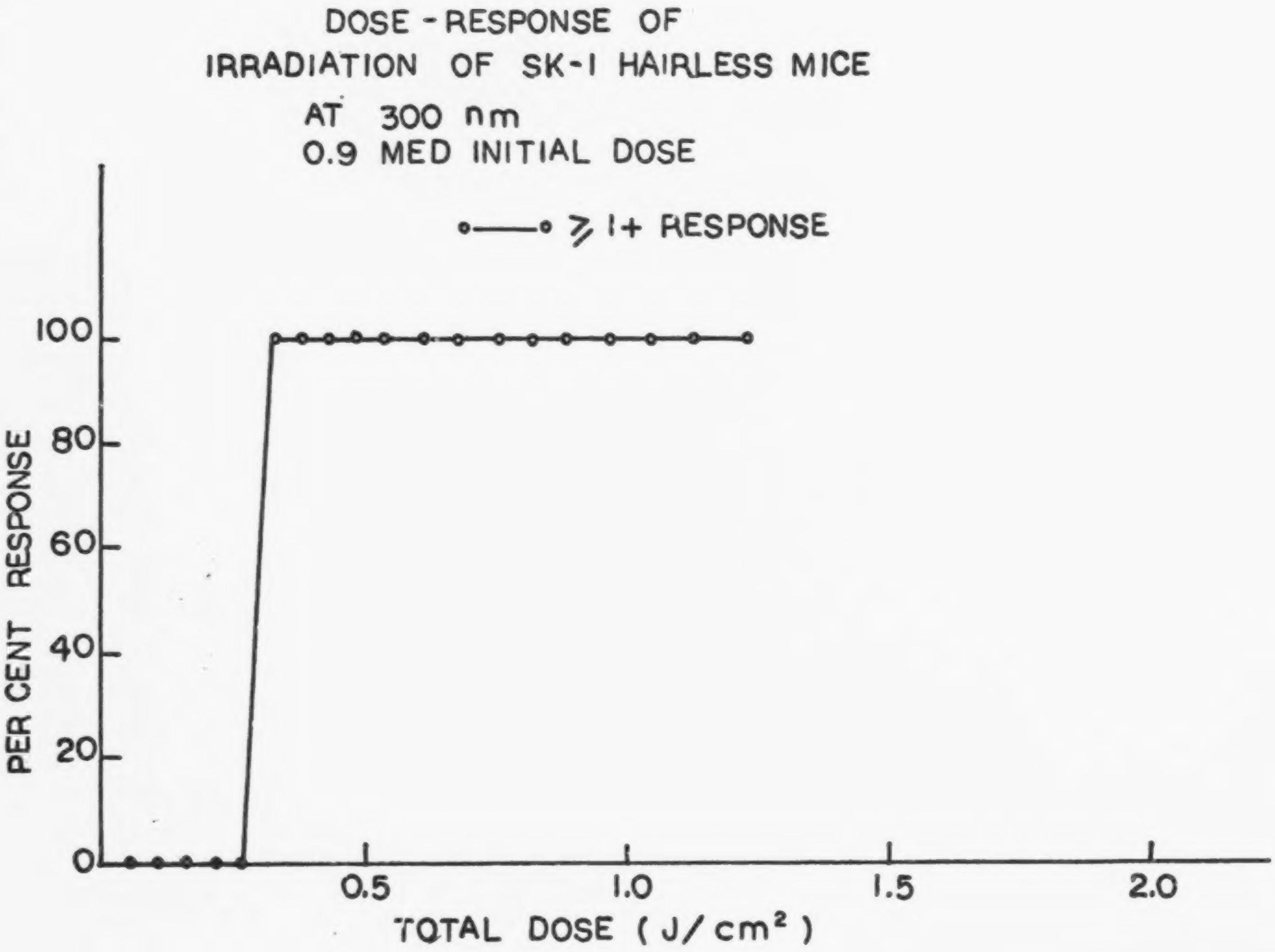


Figure 14

761

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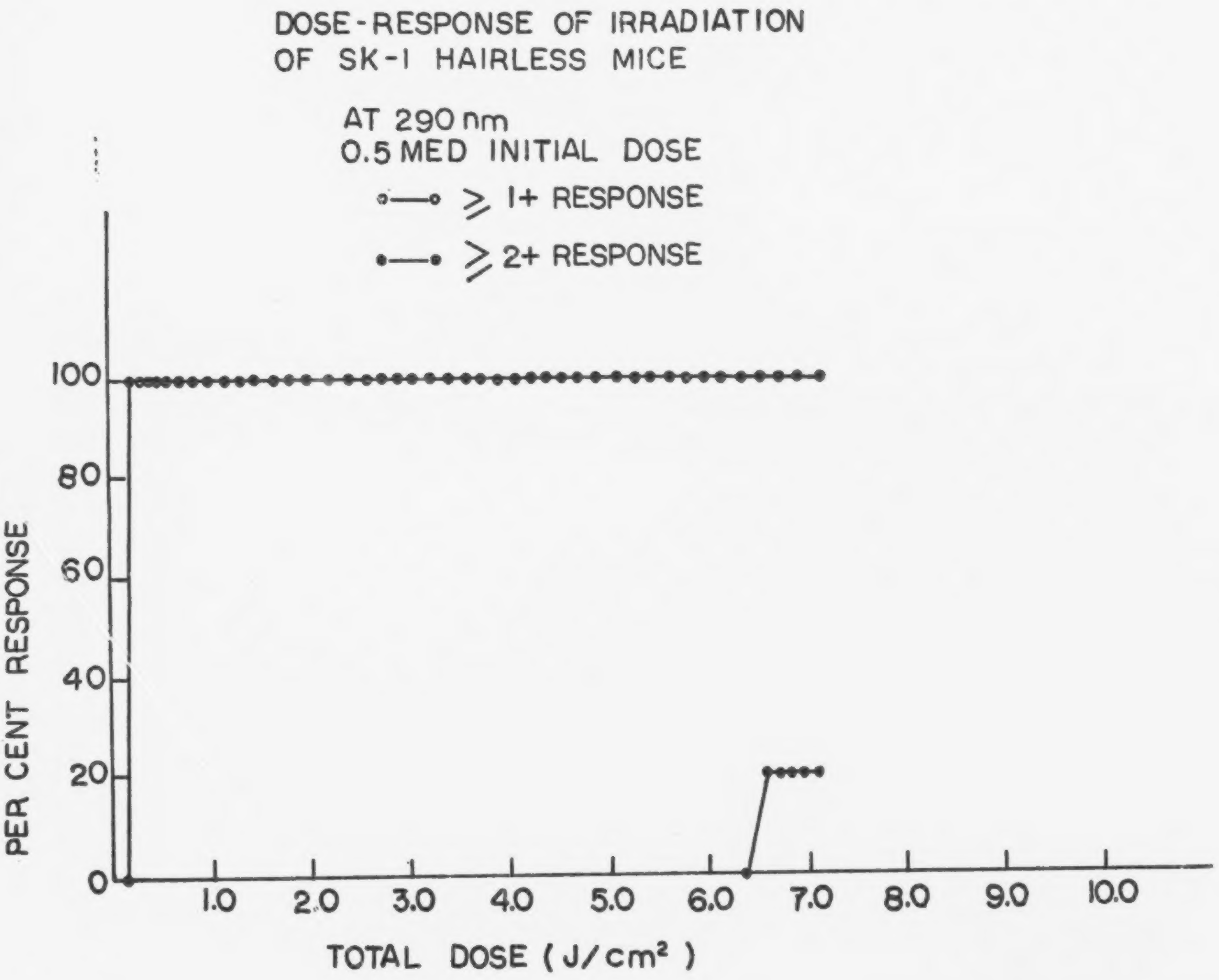

Figure 15

762

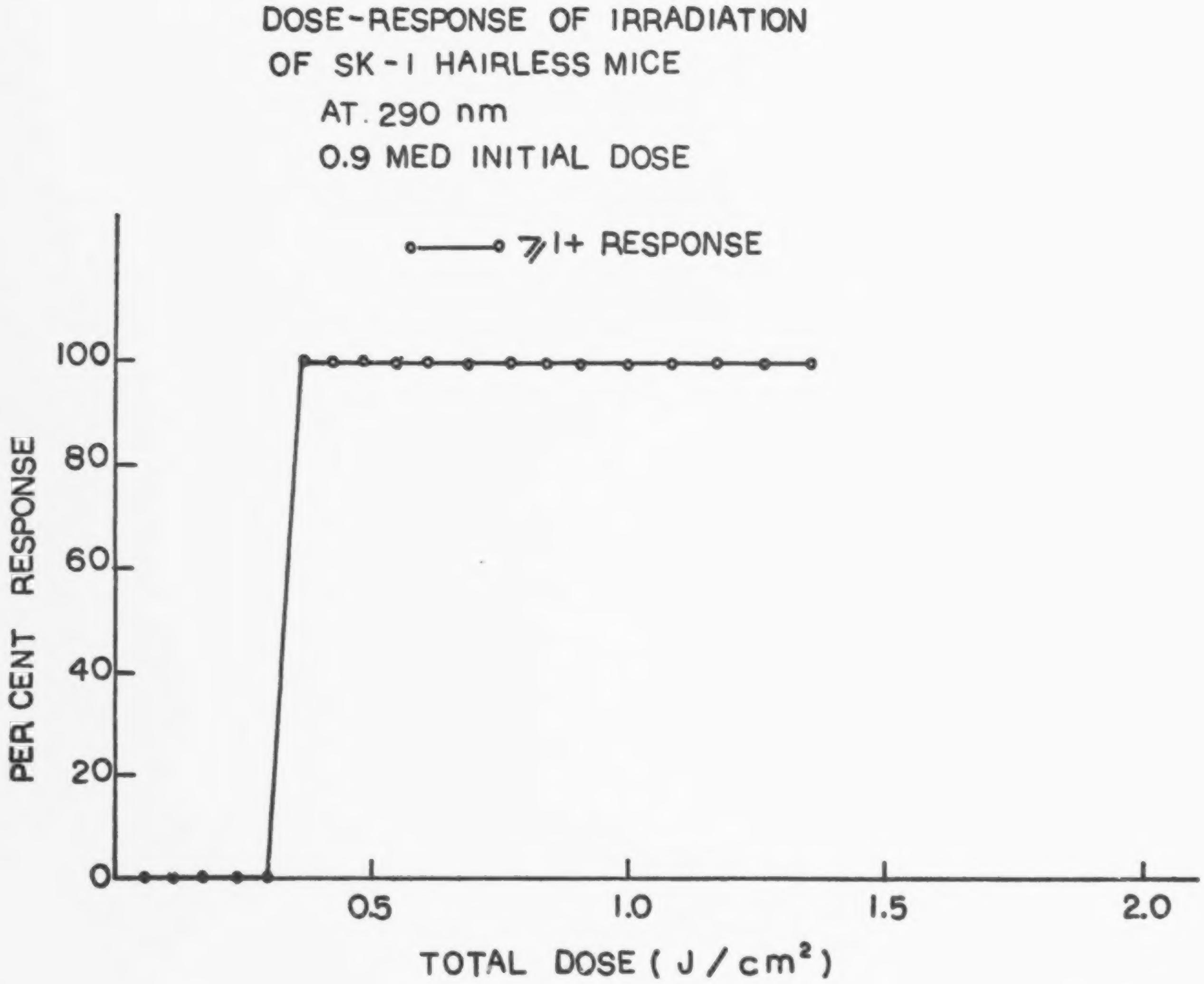 DOSE-RESPONSE OF IRRADIATION OF SK-1 HAIRLESS MICE AT 290 nm 0.9 MED INITIAL DOSE

Figure 16

763

~~DOSE-RESPONSE OF IRRADIADTION~~

DOSE-RESPONSE OF IRRADIATION
OF SK-1 HAIRLESS MICE
AT 280 nm
0.5 MED INITIAL DOSE

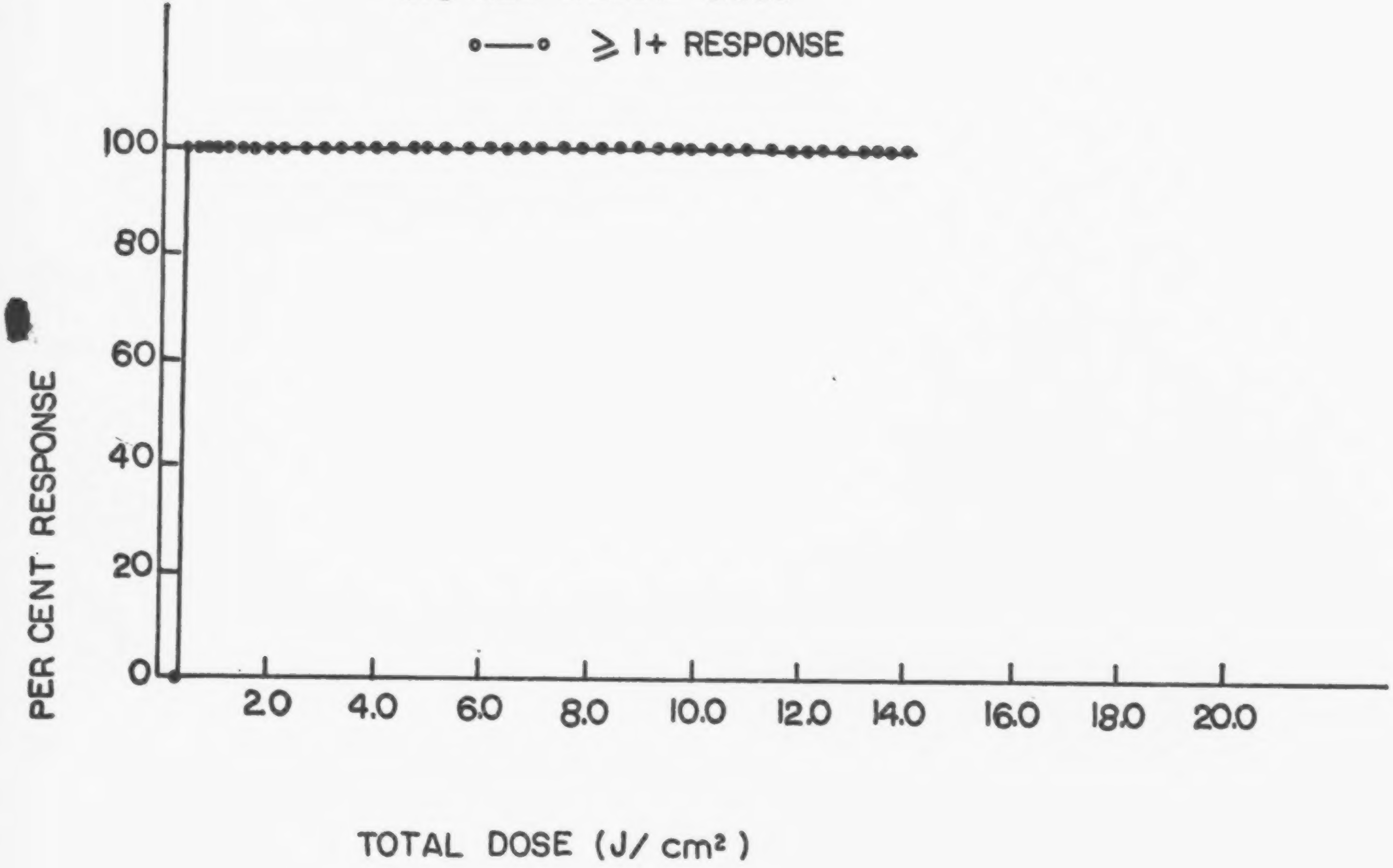

Figure 17

764

DOSE - RESPONSE OF
IRRADIATION OF SK-1 HAIRLESS MICE
AT 280 nm
0.9 MED INITIAL DOSE

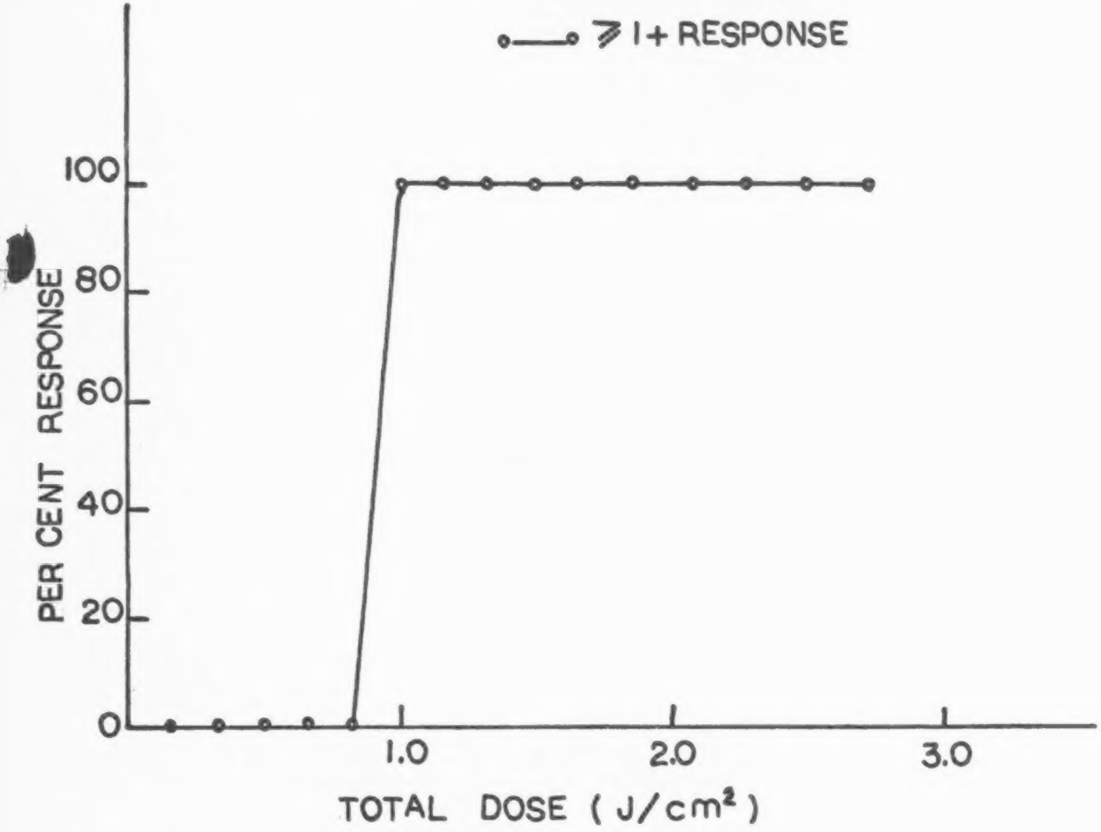

Figure 18

765

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Figure 19

ENERGY ACTION SPECTRUM FOR
1+ REACTION IN SK-1 HAIRLESS MOUSE

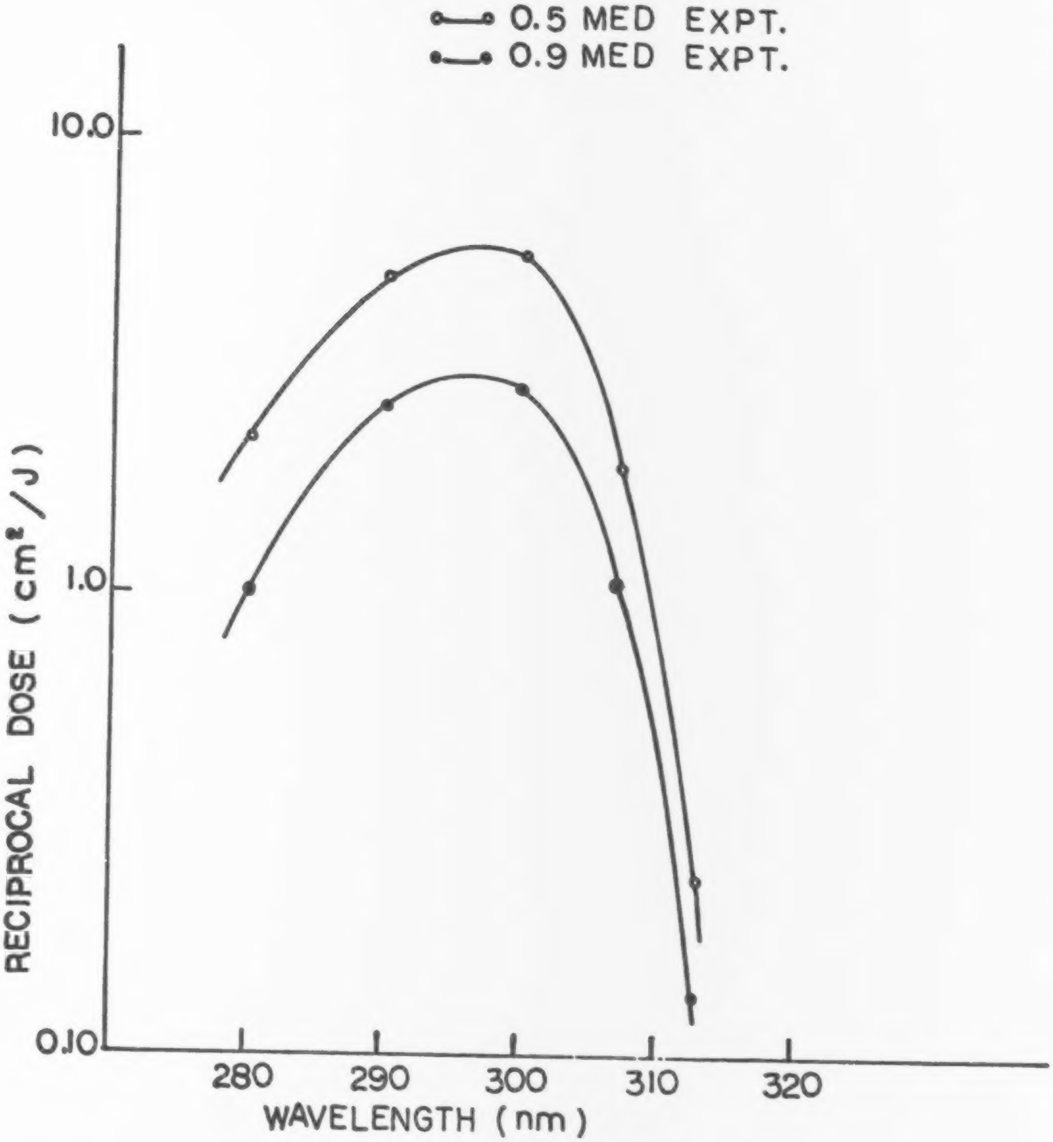
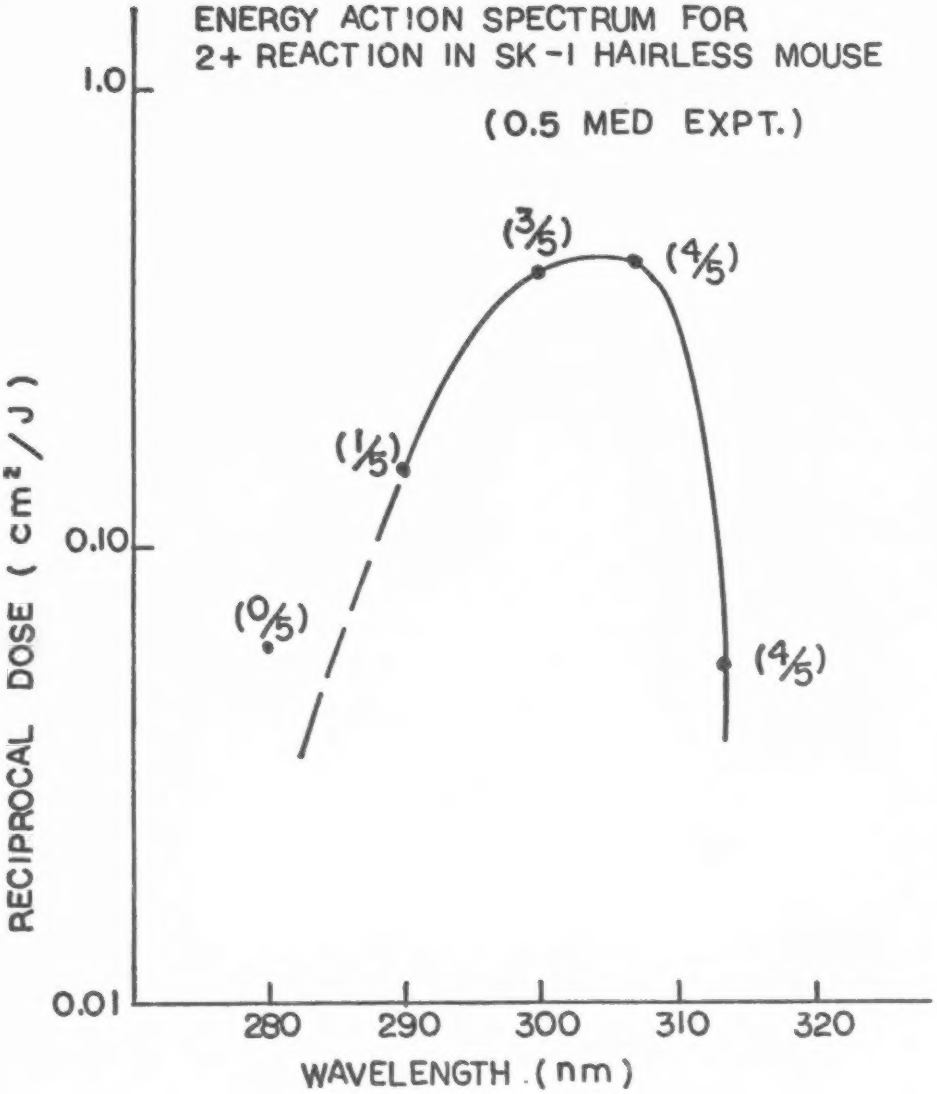


Figure 20



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PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

PRESENTATION AND DISCUSSION:

In Vivo Analysis of UV-B Induced Photooxidations

Norman I. Krinsky

Tufts University School of Medicine
Department of Biochemistry and Pharmacology
136 Harrison Avenue
Boston, MA 02111

NCI/EPA/NIOSH Second Collaborative Workshop: Progress on Joint Environmental and Occupational Cancer Studies, September 9-11, 1981.

IN VIVO ANALYSIS OF UV-B INDUCED PHOTOOXIDATIONS

Norman I. Krinsky

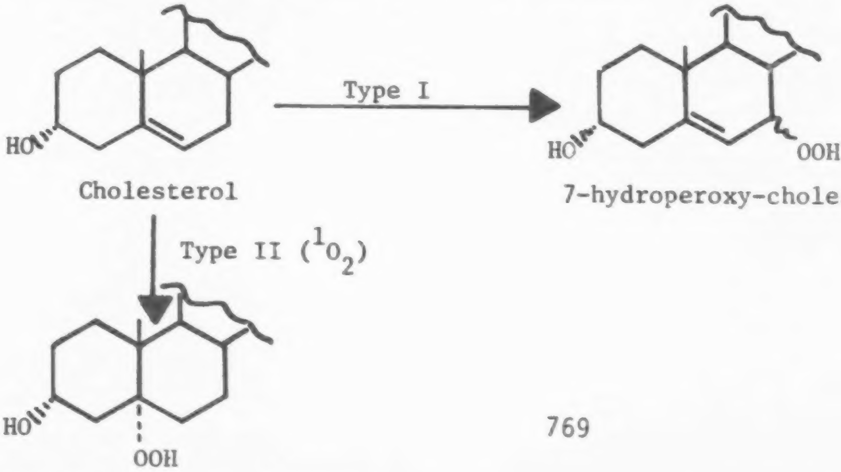
Department of Biochemistry and Pharmacology
Tufts University School of Medicine
Boston, MA 02111

Although little doubt exists regarding the significance of UV-B in initiating tumor production in experimental animals and man, the mechanism of this photobiological phenomenon is not clearly understood. UV-B has been shown to act both as a tumor initiator and as a promoter of chemically-induced carcinogenesis. Again, it is not at all clear whether these actions are initiated at the level of DNA modification, or if they occur via lipid peroxidation or through the generation of active oxygen species. The aim of this project is to clarify the initial photochemical mechanisms associated with UV-B irradiation *in vivo*.

To achieve this objective, we plan on taking advantage of our knowledge of the photochemical and oxidative reactions of cholesterol, an important constituent of biological membranes. When cholesterol undergoes photochemical oxidation it yields products that represent either a Type I or Type II photochemical reaction. Type I and Type II photochemical reactions arise from either radical processes or singlet oxygen reactions as described below, where S is a photosensitizer, R is a reactant capable of electron or hydrogen abstraction and $^1O_2$ is singlet oxygen, an electronically excited species of molecular oxygen, which is found in nature as a triplet state molecule, $^3O_2$.

$$S \xrightarrow{h\nu} {}^1S \rightsquigarrow {}^1S \begin{cases} \xrightarrow{R} S^{+\cdot} + R^{-\cdot} & \text{Type I} \\ \xrightarrow{^3O_2} S + {}^1O_2 & \text{Type II} \end{cases}$$

The reaction products expected for cholesterol, when either a Type I or Type II photochemical reaction occurs, are shown below:



Cholesterol

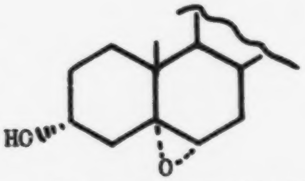
7-hydroperoxy-cholesterol

5α-hydroperoxy-cholesterol

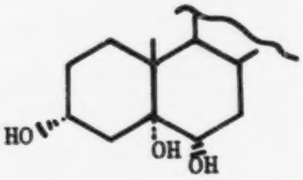
The Type I products are the epimeric 7-hydroperoxy-cholesterols. In the case of the Type II reactions, the product is 3β-hydroxycholest-6-ene-5α-hydroperoxide. These products can be readily separated from each other by thin-layer chromatography (TLC) or by high performance liquid chromatography (HPLC). However, when only small amounts are to be analyzed, it is necessary to use radioactive cholesterol as the starting material and analyze the radioactivity in the various products.

We have been using the hairless mouse, Skh-hr-1, as our test system for evaluating the primary process of UV-B photooxidation *in vivo*. In order to produce radioactive cholesterol *in vivo*, the animals are injected with ^{3}H-mevalonic acid one hour before the initiation of the UV-B irradiation. Under these circumstances, the major radioactive lipid formed is cholesterol. After suitable UV-B irradiation, the animal is sacrificed, the skin isolated and lipids extracted. A preliminary TLC separation leads to the isolation of a sterol fraction which is resolved by HPLC. Preliminary experiments indicate that UV-irradiation leads to the formation of a compound(s) more polar than cholesterol but the quantities produced have not as yet permitted an adequate identification.

However, products other than the hydroperoxides can also be expected and will be investigated. For example, cholesterol-5,6-epoxide and its product, 3β,5α, 6β-trihydroxycholesterol have been reported in skin supplemented with radioactive cholesterol and irradiated with UV-B *in vitro*. Interestingly, these compounds can also be identified when radioactive cholesterol is incubated *in vitro* with white blood cells such as polymorphonuclear leukocytes (Krinsky, unpublished observations) or when the radioactive cholesterol is exposed to gamma-irradiation *via* a ^{60}Co source (Smith, L.L., 1981). These reaction products are described below:



5,6- α -epoxycholesterol



3β -5α-6 β -trihydroxycholesterol

This technique described above will be extended to include studies on the mouse strain, Skh-crh, which has a low incidence of UV-B induced skin tumors in order to compare the initial photochemical oxidation products with those observed in the Skh-hr-1 strain, which has a high incidence of tumors following UV-B irradiation.

DR. KELSEY: Dr. Krinsky, from my reading of the literature, the alpha-oxide has never really been shown to be conclusively a carcinogen. My question is, are any of these other photooxidation products planned to be tested? Or do you know if they have been tested in animal systems?

DR. KRINSKY: Well, I know that the alpha-oxide has not been effective in the Ames salmonella microsomal system. I must confess, I don't know if other cholesterol oxidation products have been tested and we, ourselves, are not planning on doing any of that particular work.

DR. KELSEY: I believe that Black had initially gotten everybody excited, but I think in going back they have done some studies and it doesn't seem to be very effective unless there is some other promoting agent used. And even those experiments look at little bit, you know, kind of shaky.

DR. KRINSKY: Yes, the interest has waxed and waned. It waxed with Black's work and then waned. And it has partially waxed again because of Kelsey and Pienta.

DR. KELSEY: Well, I think it is a very interesting finding, but the proof is really going to be if we can do it in animals.

PROCEEDINGS OF THE SECOND NCI/EPA/NIOSH COLLABORATIVE WORKSHOP: PROGRESS ON JOINT ENVIRONMENTAL AND OCCUPATIONAL CANCER STUDIES

Friday Afternoon, September 11

PLENARY SESSION

Session Chairperson:
Dr. Richard H. Adamson
National Cancer Institute

DR. SAFFIOTTI: Any other questions? Thank you very much. With this we bring to a close this portion of the afternoon's session, and I would now like to turn over the chair to Dr. Adamson, Director of the Division of Cancer Cause and Prevention at the National Cancer Institute for the plenary session. Dr. Adamson.

DR. ADAMSON: Rather than have a panel of the chairpersons and the discussion group people come up here since the audience is so sparse, I think that I will just ask the people to stand and make comments into the microphone.

Before I ask the various chairpersons to summarize their comments or recommendations I would like to make a couple of points myself.

First of all, I would like to thank the organizing committee and the contractor involved, and everybody that stayed until the last time period here. Secondly, I stated on Monday night, the opening night, I do feel that these workshops are useful but I do feel that the annual workshops are too frequent and perhaps we should hold a third joint workshop perhaps two years from now.

There are various improvements that can be made with regard to the mechanics of conducting the meeting. Dr. Kraybill has sounded off on one of these. I must say, that slides this afternoon have been much improved over some of those this morning. Perhaps we need to have the contractor develop a workshop on how to present data on slides. Some of those slide presentations were so complex that not only could they not be seen, but the point was entirely lost from the back of the room.

I do think we could make use of a laser pointer or a light pointer in any future workshop so that a person can stand here and not have to walk away from the microphone.

And I think the program can be better organized so that work that is in progress can be organized into one area, and work that is completed can be organized in another. And we can give more time allocation to work that is completed, and less time to work that is in progress -- perhaps ten minutes and 20 minutes.

And perhaps, if we have a workshop a couple of years from now, we should think of having an overall discussion by one person in the realm of occupational carcinogenesis, or epidemiology, or some sort of a theme, so that we have one key address by somebody.

One additional point, that is, the mechanism of the pass-through money, perhaps it is time for both agencies, both NIOSH and EPA, as well as NCI, to think of using some of this money in areas of putting out an RFA, a request for announcement, through the National Cancer Institute, in areas of mutual interest so that we have some grant money -- instead of all the pass-through money going to contracts. This, obviously, would have to be in areas of mutual interest but we would set up a special ad hoc committee to review the grants in the areas of either occupational or environmental exposure on which we could mutually agree.

Those are the only comments I have. One additional thing, any recommendations anyone has to make, if they can send these or if they can scribble them down now on a piece of paper and give them to Dr. Kraybill, or have them typed and sent to him, will then be typed up and we will have the committee from the three agencies look at them and make their recommendations to me. I will look at them and pass them on, and perhaps we can put them in with the publishing of the proceedings.

Now I would like to ask the various chairpersons and group leaders if anyone has any comments, recommendations, or conclusions to make.

DR. SAFFIOTTI: My main interest in this portion of the program is in the fact that there are some areas in which the epidemiology approach and the experimental laboratory approach are beginning to move closer together. Their cooperation is a goal we have talked about for many years but which is still somewhat elusive. It takes an effort on the part of both sides for it to be consolidated.

There have been indications of studies in which laboratory evidence has brought epidemiologic studies to focus on particular problems with useful data, and areas in which epidemiological suggestions have been followed up with laboratory studies.

Some important points that came up during discussions on laboratory methods concern the variability of results, the definition of laboratory model systems, and the question of reliance on one or more of them. This point was discussed. I think we need to look at batteries of biological systems for several types of studies to avoid the possible one-sidedness of single biological systems.

What comes out of this discussion is an expanding matrix that we need to fill. We have a continuously growing number of biological model systems that can be used for these types of studies; some of them are more appropriate for testing, but many of them, fortunately, are becoming more extensively used for mechanism-oriented studies.

But one can visualize a situation where you have many biological models and hundreds of substances that you want to study, plus combinations of doses and interactions: obviously, one cannot easily embark on such a systematic effort. This means that we have to develop criteria for selecting our priorities in the choice of biological systems as well as of substances for study. At present, there is a certain scattering of activity. Any of the combinations of a different chemical in different biological systems can generate data on which to write papers, but we need to select the most meaningful and effective studies.

What is the best way for the three agencies, assisted by the advice of experts from the scientific community at large, to utilize their resources in a constructive effort in this area? From the laboratory point of view, a challenge for the next few years is to try and identify major directions of fruitful research in the development of these programs. Otherwise we could easily scatter resources in producing data that could be accurate but that would not add very much to our ability to make solid evaluations of the major problems in environmental and occupational cancer. I suggest that we could give higher priority to research addressed to those areas in which we see the most promising convergence of data relevant to biological significance, instead of first using relatively few biological test systems -- some in vivo and some in vitro and running a lot of chemicals through them. It might be more useful, at this stage to take a relatively limited number of chemicals that look promising by a variety of criteria and then explore not only their bioassay response, but also their mechanisms in a variety of biological systems related to the human end points.

I recommend that we should try to have more critical planning of the direction of this whole joint effort rather than letting it develop in possibly interesting, but somewhat scattered areas.

DR. ADAMSON: Those are very useful comments, particularly in light of the fact that we only have so much in the way of resources and, obviously, we cannot just look at all types of complex mixtures just randomly.

The point with regard to linking epidemiology and laboratory studies actually was made yesterday at the plenary session in one of the summaries. And it is something that we have wanted to do at NCI for a while. And that is one reason for the creation of the biochemical epidemiology section at the National Cancer Institute. Dr. Weisburger, do you have some comments or recommendations you want to make?

DR. WEISBURGER: Although we had only a short discussion session, I think the one point that came out of it was that mixtures are what we are really exposed to out there in the world, and it is time to start looking at that a bit more seriously. And a good thing, as Dr. Saffiotti said, would be to try some of these batteries of short-term tests. At least it would be a start. It is too big a problem.

DR. ADAMSON: No, I agree. I didn't mean to suggest that we should throw up our hands in horror. I just think we need some planning before we get into the mixtures because, obviously there are so many that we can do mixtures from now until we run out of money.

So I think in order to get into looking at mixtures, both with regard to possible potentiation, additive effects, or amelioration effects, that we need to have some sort of serious discussions with regard to that. Dr. Galbraith, do you have any comments?

DR. GALBRAITH: Well, I would like to reiterate what Dr. Saffiotti said. In addition, I believe it would be a good idea for projects in progress, i.e., newly initiated projects, that the presenters give a much more thorough justification regarding why the projects were initiated.

Statements like, there are indications of an increased rate of tumor, or a greater hazard, are not appropriate for newly initiated studies for which results are not yet available. I would have liked to have learned the epidemiological justification for some of the mechanistic studies that were presented.

A sound justification is particularly important in view of the lack of research funds available to all agencies. I agree with the comment that the NCI/EPA mechanism needs a clearer focus. The resources of all the agencies involved are constantly changing. And it might be a good time to take a look at our resources and determine how this mechanism can fill the greatest need in the future.

DR. ADAMSON: Dr. Spirtas?

DR. SPIRTAS: We have had some similar discussions in the epidemiology session. Dr. Bridbord brought up the same point of multiple contributing causes in epidemiologic studies and the point that because a study shows an association with a certain chemical should not preclude us from looking more deeply into other possible causes.

Along the same lines, Dr. John Cooper has expressed an interest in exposure dictionaries, or ways of relating which exposure(s) are linked to specific jobs within industries. There is to be a session next spring, sponsored by Dr. Acheson in Great Britain, to address this point.

Another point brought up by Dr. Austin was the lack of adequate legislation regarding medical surveillance. He brought up the example of a company which is afraid to examine its employees because if they find positive results they may be sued. He sees no adequate way of handling this, other than legislation.

Along the same lines, I brought up the point that the General Accounting Office is looking at the possible ways of changing legislation to allow researchers greater access to Social Security and Internal Revenue Service files for epidemiologic studies. This approach has been explored by NIOSH most recently.

This tied in with a point that Dr. Burton made about the difficulty of tracing cohorts, where we have to go to contractors who go through elaborate mechanisms and spend quite a bit of money to go to commercial credit bureaus to trace people when the same information is available cheaply within the government. We, as well as other government researchers and university researchers, don't have access to the information that our government keeps. But we have to do things that are more invasive of people's privacy, and more expensive, because of the current legislative situation.

So, one breakthrough that we see in the field of epidemiology would hinge upon legislative efforts, and not simply scientific efforts, but our willingness to talk to the legislative people and the people in other areas of government who are pushing to relax or to change our access to data.

DR. ADAMSON: Thank you. Dr. William Farland?

Dr. FARLAND: I guess I would just reiterate a few of the points that Dr. Saffiotti made. Very briefly, our discussion suggested the need for additional information, either increases in the development of methodology or new applications that would provide background for interpretation and strength for extrapolation of epidemiology studies. I think that was one of the main things that come out of the discussion.

One of the points that did come up specifically, that I think was of interest, was the fact that we have some exciting methodology presented in these papers, and if we are to apply the hybridoma work not only to a clinical and therapeutic type of a situation, but to an identification type of a situation, one would like to be able to make sure that we develop additional information from individuals who are being screened so that we would be able to explore some links between environmental exposure and some of the health effects that are being detected.

Once again, I will reiterate the recommendation that we explore the use of batteries that would be multifaceted, included metabolism work, various types of short-term in vivo and in vitro tests, bioindicators, all of those sorts of approaches in coming to a rational scientific decision with regard to human hazards.

And I would restate as a final suggestion that perhaps we need to explore additional means for using the situations that we have available to us to extend our collaborative efforts into the sectors of academia and industry.

DR. ADAMSON: Are there any comments from anyone else in the audience that would like to say something?

DR. HELLMAN: There are a couple of things that came to my mind. First of all, I was rather appalled somewhat in the duplication of many of the experimentations going on in this group and other areas that I am aware of, doing very similar work. In other words, with the shortage of funds you would think that this would have to be sort of gleaned out a little better.

The other thing which kind of concerns me is the idea of continually doing more and more testing for carcinogens. I mean, it is a never-ending problem obviously. I wonder if one might not look at it a little the other way, namely, start as the industrial plants are being refurbished, and they have to be in view of the problems that we are having competing with the rest of the world, we ought to maybe start thinking about putting environmental controls at that phase, with the idea that if you can eliminate exposure, or minimize exposure levels, then many of these problem areas that we are currently faced with may be also reduced appreciably. I guess that is basically it.

DR. ADAMSON: Yes, actually, there are a number of -- I guess it is all right to mention one -- Dupont, certainly with regard to a lot of the new plants, it puts in very good control devices instead of having to go back and do retrofitting. I am sure that Ken Bridbord could name a number of other plants.

But certainly the large industrial chemical plants are well aware of the hazards and are taking adequate precautions, at least in my estimation, for protection. And I don't think it is the great large new plants that are problems to us. Ken?

DR. BRIDBORD: I would like to follow these recent comments. I agree very much with their thrust, with a major, major need in terms of engineering controls. That is really curriculum development for engineers. The basic education process for engineers is lacking, particularly in terms of control technology issues for the work place because the concept of new source performance standards and technology to protect workers against exposure is really not anything that is included in the curriculum. And if we could somehow change that social mix in terms of that sensitivity to the problem, that would be a very important factor. And that is one of the items on our agenda, hopefully, to chat between NIOSH and NCI so some more can be done.

DR. ADAMSON: Sort of like trying to work nutrition into an agenda during medical school, which is very difficult in this country. Are there any other statements? Yes.

DR. FORBES: One brief comment and a plea that is related to the kind of comment that says let's look at mixtures which is more like real life, and to add to that let's not pretend that dark chemistry is the only thing that occurs. Life as we know it could not have evolved in the dark and we do not live in the dark. A great deal of photochemistry is happening all around us.

So we should be more aware, I think, of the involvement of light with the chemical phenomena that we are concerned about. Specifically, it has been known that a number of carcinogens could be either decreased or increased in effectiveness by the ambient light to which they are exposed. And at least one of the projects discussed today involved light interaction with chemical effects.

But it is not just a matter of whether there is light there, but when it is there, before, during or after the chemical exposure. Now, this is not just a plea for funds for photobiology, it is a plea that when projects are discussed and undertaken that those who are available who know some photochemistry be involved in such planning so that photochemistry and photobiology can be taken into account. The society known as the Society for Photobiology, I am sure has a number of people that would be glad to help in that kind of planning.

DR. ADAMSON: Thank you. Certainly, there is data available that indicates -- with regards to some of the organic pesticides, that there certainly is a difference with regards to their persistence in the different latitudes and with regards to whether they are near the equator and how much sunlight and how much humidity there is. That is one example.

Certainly, another example is with regards to exposure to the PCB's and their break down or lack thereof, with regards to esposure to sunlight. So I think your remarks are well taken.

Any other comments or questions. Herman, do you want to have the last say here?

DR. KRAYBILL: I think I have said enough the last couple of days and I told myself I wasn't going to say anything. But Dr. Hellman raised my interest. I was in the PHS pesticide program and I will address myself to that point. Sure, company "A" -- and I won't mention which company -- I know some good ones have done a lot to reduce the exposure to the worker. When you are going in to do an epidemiological study there are those companies where there is minimal exposure.

But lest we forget, we cannot ignore those substances that stay around for a long while -- chlordane we used to treat for termites and DDT for other purposes, you can name them. The women up in Michigan are still spilling out in their mothers' milk DDT, and DDT was banned a long time ago. And in mothers' milk is PCB and I guess now PBB. So, those chemicals are going to be around. Therefore, we have to think of those long-term exposures even though we banned them and the plant doesn't use them any more; although they are no longer allowed, they are going to be in people exerting an effect at the cell level.

DR. ADAMSON: Thank you. Any other comments? I hereby declare the workshop adjourned. Thank you very much.

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